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PROGRESS

THE VERIFICATION TEST IN THE SEROLOGY OF SYPHILIS*

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INTRODUCTORY

1. *The Verification Test—The Search for Characteristics Which Differentiate Specific From Nonspecific Serologic Reactions.*—Studies in progress in this laboratory aimed at the detection of differential characteristics of true and false positive serologic reactions have thus far led to two observations which have afforded a basis for several practical procedures. The observations are that, within certain limits, (1) specific serologic reactions tend to become stronger and nonspecific reactions weaker as the reacting temperature is increased; and (2) specific serologic reactions tend to become stronger and nonspecific reactions weaker as the salt concentration of the reacting system is increased. The practical procedures are (1) differential temperature technique, (2) triple quantitative technique, and (3) salt dispersibility technique.

2. *Occurrence of Biologic False Positives.*—The nature of biologic false positive reactions is still obscure. It is believed that the following two factors largely determine the occurrence of these reactions with serodiagnostic tests: (1) a tendency to give such reactions on the part of certain individuals, and this tendency may be constitutional in nature; and (2) some pathologic disturbance (fever, cancer, etc.) or biologic imbalance (pregnancy, vaccination, etc.) serving as the immediate cause of such reactions, although the reactions are encountered also in individuals apparently free from pathologic disturbance or biologic im-

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This article is not a review on false positives and their detection. The article considers only the present status of the verification test. The experimental studies upon which this article is based were carried on in this laboratory in association with Elizabeth B. McDermott, Jacob Adler, Stanley Marcus and other members of the staff, and will be published elsewhere.

balance. The occurrence of positive reactions in the absence of syphilis is unique in that many individuals who show no tendency to give these reactions with serodiagnostic tests begin to give them with the increase in the sensitivity of the tests, indicating that seronegative persons possess latent potentialities for manifesting such reactions.

3. *Relation Between Biologic False Positives and Serologic Reactions Given by Lower Animals.*—Studies carried out in this laboratory indicate that false positive reactions given by human beings and the positive serologic reactions given by pigs, horses, rabbits, and other animals possess similar characteristics. Thus, these two types of reactions are most marked (1) when the tests are performed at cold temperature (1° C.), and (2) when the salt concentration of the reacting system is reduced to a minimum. These findings suggest that a close relationship exists between human and animal reactions of nonsyphilitic origin given by serodiagnostic tests. In this laboratory, indeed, animal sera are commonly used for studies of false positive reactions.

4. *Basis of Biologic False Positives.*—Tissue extract antigens of excessive sensitivity can be prepared which will give about 40 per cent of positive serologic reactions in nonsyphilitic persons. If the serologic tests with excessively sensitive antigens are performed at cold temperature, the nonspecific sensitivity can be raised to about 80 per cent. If the tests with excessively sensitive antigens are performed at cold temperature with unheated sera (instead of with sera heated for 30 minutes at 56° C.), the nonspecific sensitivity reaches about 98 per cent. Certain lower animals (horses, pigs, chickens) also give 100 per cent of positive reactions when the tests are performed under the same conditions. These findings suggest that there exists a "universal" serologic (nonsyphilitic) reaction, given by human beings and certain lower animals, which is best manifested under conditions of low temperature. It was shown before that the biologic false positive reaction given by serodiagnostic tests is similarly best manifested under conditions of low temperature. Hence, it would seem reasonable to assume that the two reactions are related to one another and that the universal reaction may be the basis of biologic false positives.

5. *Biologic False Positives in the Presence of Syphilis.*—Biologic false positives may be given by persons with syphilis. Thus, a certain person has a tendency to give false positives, let us say, when he contracts a cold. In due time, he becomes infected with syphilis and he begins to give specific reactions. Then, when he contracts a cold, he is likely to give at the same time both specific and nonspecific reactions. Should he become specifically seronegative following therapy, he will give only the nonspecific reaction when from time to time he has a cold. Also, certain neurosyphilitic patients treated with malaria give specific serologic reactions before malarial therapy, both specific and nonspecific reactions soon after the therapy, and in due time only specific serologic reactions again.

6. *Technical and Biologic False Positives.*—False positives are generally classified as either technical or biologic, but it is not always possible to draw a line of demarcation between them. A false positive given by a supersensitive test, for example, may be interpreted by some as technical, because the antigen

in this test shows a tendency to give, let us say, 5 per cent of false positive reactions. But the fact that 5 particular persons in 100 gave these reactions would indicate that the sera in these persons differed biologically (biochemically or physiochemically) from the sera in the remaining 95 persons, thereby rendering these reactions biologic false positives. Experience indicates that any serodiagnostic reaction suspected of being a false positive, whether biologic or technical, may be investigated with the verification test.

7. *Verification Results and Clinical Findings.*—The dependability of laboratory results in a given infection is generally determined by the extent to which these results are corroborated by clinical findings of that infection. Unfortunately, verification reports of false positives cannot be corroborated by a definite clinical indicator. Negative clinical findings of syphilis represent *the* clinical indicator of false positives, and negative findings cannot have the same value as positive findings. Yet, in the diagnosis of false positives, the establishment of negative clinical findings of syphilis is obviously of first importance. When such findings corroborate verification results of false positives, the significance of these results immediately becomes fortified, just as the significance of positive Widal reactions becomes fortified by clinical findings of typhoid fever. The verification test, like other laboratory methods of an immunologic nature, does not lend itself to mass testing. Even with serodiagnostic tests having a record of use for over a generation, mass testing is acceptedly wrought with difficulties. Briefly, in the application of the verification test to the detection of a false positive reaction in a given case, it is assumed that thorough search for clinical evidence of syphilis had led to negative findings and that the examining physician had concluded that the patient was most likely free from syphilis. A verification report of a false positive reaction in a case such as this may thus be said to be corroborated by clinical findings.

THE "TYPING" OF SERODIAGNOSTIC REACTIONS

8. *Reporting Verification Results to Physicians.*—The function of the verification test is to type positive serodiagnostic reactions on the basis of their specificity. Hence, the results of the verification test are reported not as positive or negative, but only in respect to the specificity of the serodiagnostic reactions. To illustrate: (1) A physician receives a report of a positive serodiagnostic reaction in a given case. (2) He believes this reaction to be a false positive and requests a verification test. (3) The verification test determines whether the serodiagnostic reaction shows characteristics of a luetic type or of a nonluetic type of reaction or perhaps of both types.

9. *Types of Serodiagnostic Reactions Encountered With Verification Test.*—(1) "General biologic (nonluetic) type"* is the designation employed when, according to the verification test, a serodiagnostic reaction shows characteristics of a false positive. (2) "Type of reaction similar to that obtained in lues" is the designation employed when, according to the verification test, a serodiagnostic

*This designation, it is believed, is more desirable than the purely negative designation of "false positive," since in addition to indicating absence of syphilis it indicates the presence of a reaction which in many instances accompanies a pathologic disturbance or biologic imbalance. The term "general biologic" is being employed because of the biologically widespread nature of this reaction, especially among lower animals. The term we believe is also preferable to "biologic" in designating false positives, since syphilitic reactions or "true positives" are also biologic reactions.

†The terms "lues" and "luetic" are employed instead of syphilis and syphilitic because the latter terms are too obvious to the laity.

reaction shows characteristics of a syphilitic reaction. (3) "Negative type" is the designation employed when the results of the verification test are negative. (4) "Inconclusive" is the term used when the verification results are atypical. (5) Occasionally, a positive serodiagnostic reaction embodies, according to the verification test, both the syphilitic and nonsyphilitic types of reactions.

10. *Procedures of the Verification Test.*—(1) The triple quantitative technique, described later, is employed in typing strongly positive (++++) serodiagnostic reactions on the basis of their specificity. Three types of reactions are generally encountered: the luetic type, the general biologic type, or a combination of both types. (2) The differential temperature technique, also described later, is employed in the typing of moderately and weakly positive (+++, ++, + and ±) serodiagnostic reactions. Here also three types of reactions are generally encountered: the luetic type, the general biologic type, and a combination of both types. (3) The salt dispersibility technique, due to its simplicity, is employed with all sera. (4) The standard Kahn test and a modified Kahn procedure (Method B) are also employed in performing verification tests.

THE VERIFICATION TEST IN KNOWN CASES OF SYPHILIS

11. *The Verification Test in the Various Stages of Syphilis.*—The vast majority of syphilitic cases give the luetic type of reaction with the verification test; a relatively small number give both the luetic type and the general biologic type. In a small percentage of cases of early syphilis, only the general biologic type of reaction may be encountered. In repeated examinations, as the serologic reaction becomes stronger in this stage of syphilis, the luetic type of reaction is obtained. This temporary occurrence of the general biologic type of reaction in early syphilis must be kept in mind when this type of reaction is reported in a case in which there is the slightest possibility of the presence of early syphilis. Repeated verification tests at intervals of a week or more are necessary. These tests will indicate the trend of the serodiagnostic results and will determine whether the general biologic type of reaction persists. In the various stages of syphilis other than early syphilis the luetic type of reaction is generally obtained.

12. *The Verification Test in Treated Cases of Syphilis.*—The common reaction obtained is of the luetic type. Occasionally, the general biologic type is obtained. Preliminary studies of verification reactions in treated cases have led to several observations which promise to prove of clinical value. Thus: (1) The general biologic type of reaction is occasionally encountered following treatment, in addition to the luetic type, in persons who presumably show a tendency to give the former type of reaction under conditions of pathologic disturbance. It is believed that the toxic effect of the antisyphilitic treatment may result in sufficient pathologic disturbance as to be the immediate cause of the general biologic type of reaction. If so, it is reasonable to assume that a higher degree of toxicity will call forth this type of reaction to a greater extent than a lower degree of toxicity. The verification test may thus indicate variations in toxicity of different methods of therapy. (2) The verification test in certain seropositive treated cases is capable of indicating in advance that they will in due time become seronegative. This finding is based on the

observation that the formed precipitates in verification reactions show a change in stability as these cases begin to approach seronegativity. The precipitates, at first stable and indispersible, become unstable and dispersible. The tendency toward dispersibility may become manifested weeks or months before seronegativity is reached. This capability of the verification test to indicate seronegativity in advance may be of value in the study of the efficacy of various methods of therapy. (3) The verification test in certain serologically fast cases following adequate therapy may establish that the reactions are not of the luetic type but of the general biologic type.

THE VERIFICATION TEST IN SERONEGATIVE CASES

13. *The General Biologic Type of Reaction in Seronegative Cases.*—Some persons who are clinically free from syphilis and who give negative serodiagnostic reactions, nevertheless give the general biologic type of reaction with the verification test. Indications are that these persons are "carriers" of false positives. They may be seronegative with one test but seropositive with another. Or they may be seronegative today and seropositive a week later. Then again, some nonsyphilitic persons, negative with serodiagnostic tests, are positive with supersensitive tests. Verification studies of persons giving negative serodiagnostic reactions reveal three main groups: (1) those giving the negative type of reaction, (2) those giving the general biologic type, and (3) a very small group of treated cases of syphilis giving the luetic type of reaction.

THE VERIFICATION TEST IN CASES IN WHICH FALSE POSITIVES ARE SUSPECTED

14. *Routine Applicability of Verification Test to Cases in Which False Positives Are Suspected.*—Experience gained with the verification test during the past several years indicates that the greatest value of the test lies in the investigation of the specificity of positive serodiagnostic (precipitation or complement fixation) reactions in such cases in which all clinical indications point to the absence of syphilis. When the verification test is applied to such cases, the general biologic type of reaction may be said to corroborate clinical findings, while the luetic type of reaction may be said not to corroborate clinical findings. It is with this view in mind that the interpretation of these two types of reactions is considered.

15. *Interpreting the General Biologic Type of Reaction.*—A single report of a general biologic (nonluetic) type of reaction can be interpreted as a false positive with reasonable safety, provided the donor gives a history of fluctuating or weak serodiagnostic reactions extending for some weeks or months; in the face of strongly positive serodiagnostic reactions, repeated verification studies are indicated to make sure that the results are persistently of the general biologic type. A general biologic type of reaction in the absence of a serologic history (i.e. when a person gives a positive serodiagnostic reaction presumably for the first time) should be checked preferably in a week or two before establishing a diagnosis of a false positive.

16. *Interpreting the Luetic Type of Reaction.*—A luetic type of reaction obtained in a case in which clinical indications point to a false positive requires additional clinical studies and repeated verification tests. In our experience, these

clinical and verification studies have led to the establishment of syphilis in most instances. In a few instances clinicians have expressed the belief that they were dealing with false positives in spite of persistent verification reports of the luetic type of reaction. A transient luetic type of reaction has been observed in an isolated nonsyphilitic case of postinfection or postvaccination, the reaction becoming of the general biologic type in the course of some weeks. Weakly positive serodiagnostic reactions, which according to the verification test are of the luetic type, are especially difficult to interpret. Repeated examinations at prolonged intervals are often necessary before a final diagnosis can be established.

PROCEDURES

1. Triple Quantitative Technique

Applicability.—This technique is applicable to sera which give strongly positive (++++) serodiagnostic (complement fixation or precipitation) reactions suspected of being false positives.

Technique.—The technique involves the use of three quantitative Kahn procedures: in the first, the serial dilutions of serum are made with distilled water; in the second, the serial dilutions are made with 0.9 per cent sodium chloride solution, and in the third, the serial dilutions are made with 2.5 per cent sodium chloride solution. Dilution ratios of 1:5, 1:10, 1:20, 1:30, 1:40, and 1:50 are generally ample. Standard Kahn antigen suspension (prepared with 0.9 per cent salt solution) is used in 0.025 c.c. amounts, and each of the serial dilutions of serum in 0.15 c.c. amounts. After permitting the serum-antigen mixtures to stand for three to five minutes, the tubes are shaken for three minutes in the usual manner. Distilled water or salt solution of the concentration corresponding to that used in the serial dilutions of serum is then added to the tubes in 0.5 c.c. amounts; after agitating the tubes for about ten seconds to mix the ingredients, the precipitation results are read.

Results.—Known syphilitic sera generally give a higher quantitative titer with the serial dilutions of serum with 2.5 per cent salt solution than with 0.9 per cent salt solution (luetic type of reaction). Known nonsyphilitic sera generally give a higher quantitative titer with the serial dilutions of serum with 0.9 per cent salt solution than with 2.5 per cent salt solution (general biologic type of reaction).

The quantitative titer with the serial dilutions of serum with water throws additional light on the type of the reaction in question. Thus, this titer in a luetic type of reaction is lower, and in a general biologic type of reaction is higher, than the titer with 0.9 per cent salt solution, unless both the luetic and general biologic types of reactions are present. Then, the titer with the serial dilutions of serum with 0.9 per cent salt solution is lower than either with water and with 2.5 per cent salt solution. Table I presents typical reactions given by the triple quantitative technique.

Note.—Sera of low quantitative titer are also examined in this laboratory with the differential temperature technique. The sera are first diluted with an equal amount of 0.9 per cent salt solution or with half the amount (depending on the quantitative titer given by the serum) before employing the differential temperature technique.

2. *Differential Temperature Technique*

Applicability.—This technique is applicable to sera giving moderately and weakly positive (+++, ++, + and ±) serodiagnostic (complement fixation or precipitation) reactions, suspected of being false positives.

Technique.—(1) Kahn racks with test tubes are placed in a 37° C. water bath for fifteen minutes before performing the tests. The pipettes necessary in performing the test are kept at 37° C.* (2) The serum is heated for thirty minutes at 56° C. and is then placed in the 37° C. water bath for fifteen minutes before using. (3) Standard Kahn antigen is mixed with salt solution at room temperature in the usual manner according to the titer, and the antigen suspension is immediately transferred to a Kahn tube and placed in the water bath for

TABLE I

ILLUSTRATIVE RESULTS OF TRIPLE QUANTITATIVE TECHNIQUE

SERIAL DILUTIONS OF SERUM	PERCENTAGE OF SODIUM CHLORIDE SOLUTION EMPOLYED IN PREPARING THE SERIAL DILUTIONS OF SERUM		
	0*	0.9	2.5
	Luetic type of reaction		
1:5	+	++++	++++
1:10	-	++++	++++
1:20	-	++	++++
1:30	-	-	++++
1:40	-	-	++++
1:50	-	-	-
1:60	-	-	-
	General biologic (nonluetic) type of reaction		
1:5	++++	++++	±
1:10	++++	++++	-
1:20	++++	±	-
1:30	++++	-	-
1:40	++++	-	-
1:50	++	-	-
1:60	-	-	-
	Luetic and general biologic (nonluetic) types of reactions		
1:5	++++	++++	++++
1:10	++++	+	++++
1:20	++++	-	++++
1:30	++++	-	++++
1:40	++++	-	++
1:50	±	-	-
1:60	-	-	-

*Distilled water. The same antigen suspension (prepared according to titer with 0.9 per cent salt solution) is employed with each of the serum dilutions.

fifteen minutes. (4) The amounts of antigen suspension (0.05, 0.025, and 0.0125 c.c.) are then deposited at the bottom of the Kahn tubes, followed by the serum (in 0.15 c.c. quantities), and the mixtures shaken for ten seconds without removing the rack from the water bath. The rack remains in the bath for ten minutes and is then shaken for three minutes at room temperature. (5) Physiologic salt solution kept in a 37° C. water bath is then added to each tube in the usual amount (1, 0.5, and 0.5 c.c.); the tubes are agitated sufficiently to assure proper mixing and the results are read immediately. (6) A microscope mirror or a slit lamp is used in the reading of results.

*Specially constructed water baths for this technique are supplied by A. S. Aloe Co., St. Louis.

The technique at 1° C. requires a refrigerated bath at 1° C., and if not available, an ice water bath with chopped ice. The steps in the technique as carried out in the 37° C. water bath are applied to the technique in the 1° C. water bath. The temperature of the serum and antigen suspension is brought to 1° C., and the serum is added to the antigen suspension and mixed at this temperature. Tests performed at 1° C. require special care in reading the results due to the film of moisture condensed on the outside of the tubes. This film disappears by dipping the tubes in a solution of cold alcohol. Not more than ten tests should be carried out at any one time, either at warm or cold temperatures.

Results.—Syphilitic sera generally give more marked precipitation at 37° C. than at 1° C. (luetic type of reaction), while nonsyphilitic sera generally give more marked precipitation at 1° C. than at 37° C. (general biologic type of reaction). Typical illustrations of these types of reactions are presented in Table II. It is noted that similar precipitation results at all temperatures are considered as inconclusive.

TABLE II

ILLUSTRATIVE RESULTS OF DIFFERENTIAL TEMPERATURE TECHNIQUE

REACTING TEMPERATURE								
37° C.			21° C.			1° C.		
TUBE 1	TUBE 2	TUBE 3	TUBE 1	TUBE 2	TUBE 3	TUBE 1	TUBE 2	TUBE 3
			Luetic type of reaction					
++++	++++	++++	-	++++	++++	-	±	++
			General biologic (nonluetic) type of reaction					
-	+	++	-	++++	++++	++++	++++	++++
			Inconclusive reaction					
-	++++	++++	-	++++	++++	-	++++	++++

TABLE III

ILLUSTRATIVE RESULTS OF SALT DISPERSIBILITY TECHNIQUE

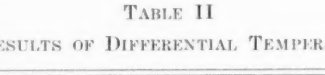
FIRST READING OF RESULTS OF STANDARD KAHN TEST IN WHICH REACTION IS MODERATELY POSITIVE

Tube 1	Tube 2	Tube 3
±	++	++++

Addition of 30% NaCl solution c.c.

0.15	0.1	0.1

Shake rack 15 seconds and place for 5 minutes in water bath at 37° C



Luetic type of reaction—precipitates are not dispersible.			General biologic (nonluetic) type of reaction—precipitates are dispersible.		
Tube 1	Tube 2	Tube 3	Tube 1	Tube 2	Tube 3
+	++	++++	-	-	-

3. Salt Dispersibility Technique

Technique 1.—When applied to weakly positive sera suspected of giving false positive (precipitation or complement fixation) reactions.

Procedure: A 30 per cent solution of sodium chloride of "reagent" quality is employed in this technique. The standard Kahn test is performed and the

results read in the usual way after the addition of 0.9 per cent salt solution to each tube. Immediately, 0.15 c.c., 0.1 c.c., and 0.1 c.c. of the 30 per cent salt solution are added, respectively, to the three tubes; the rack is then shaken by hand for fifteen seconds, placed in the 37° C. water bath for five minutes, again shaken for fifteen seconds, and the results read.

Results.—In the case of syphilitic sera, the precipitates are generally either the same as before the addition of the concentrated salt solution or they are more pronounced. In the case of nonsyphilitic sera, the precipitates become dispersed and the results appear negative. Table III presents typical reactions given by the salt dispersibility technique.

Technique 2.—When applied to all sera suspected of giving false positives and to the several verification techniques.

Procedure: Due to the simplicity of the dispersibility technique, it is being applied in this laboratory to the triple quantitative and differential temperature techniques in addition to its application to the standard Kahn technique. Thus, after reading the results of the triple quantitative technique, 0.1 c.c. of 30 per cent salt solution is added to each tube showing a precipitate, shaken fifteen seconds, incubated at 37° C. for five minutes, again shaken for fifteen seconds, and the final results read. Similarly, after reading the results of the differential temperature technique, 0.15 c.c., 0.1 c.c., and 0.1 c.c. of the 30 per cent salt solution are added, respectively, to each set of the three tubes showing precipitates and the test is completed as in Table III.

Results.—As a general rule, precipitates in nonsyphilitic reactions become dispersed on the addition of the 30 per cent salt solution, while precipitates in syphilitic reactions do not become dispersed. In early syphilis and in highly treated cases of this disease, dispersibility of the precipitates may be noted. But in the absence of clinical indications of syphilis, the dispersibility of precipitates indicates false positive reactions.

Discussion of results.—(1) *Dispersibility technique applied to four plus Kahn reactions.* A definite relationship exists between the strength of nonspecific serologic reactions and the concentration of salt solution required for the dispersion of precipitates. Since different four plus reactions may be of different quantitative titers, the precipitates in such reactions may require different salt concentrations for their dispersion. In the case of nonsyphilitic sera of high titer, precipitates which are not dispersed in the standard Kahn test by the usual dispersibility technique will often disperse by a second addition of 0.15 c.c., 0.1 c.c., and 0.1 c.c. of 30 per cent salt solution to the tubes. In some instances even this second amount of concentrated salt solution will not disperse the precipitates of the strongly positive reactions. Hence, nondispersibility of the precipitate in a strongly positive reaction cannot by itself be interpreted as a syphilitic type of reaction. The dispersibility of the precipitate is a common property of weak nonsyphilitic reactions and is occasionally noted also in nonsyphilitic reactions of high titer.

(2) *Dispersibility technique applied to the triple quantitative procedure.* Generally precipitates in syphilitic sera do not disperse on the addition of 30 per cent salt solution. Now and then a serum is encountered which gives a higher quantitative titer in the serum dilutions with 2.5 per cent salt solution than in the serum dilutions with 0.9 per cent solution (suggesting a luetic type of reac-

tion), but which shows dispersible precipitates in all tubes on the addition of the 30 per cent salt solution. It is believed that such dispersibility is strongly suggestive of a false positive reaction. In instances in which only the precipitates in the highest serum dilutions show dispersibility while the precipitates in the low serum dilutions do not show dispersibility, the reactions are generally of the syphilitic type, noted particularly in treated cases.

(3) *Dispersibility technique applied to the differential temperature procedure.* Dispersibility of precipitates in reactions obtained at 37° and 1° C. is the common picture presented by false positives. In syphilitic cases, the precipitates are generally not dispersed in reactions obtained at 37° C. but are dispersed in reactions obtained at 1° C., although they may not be dispersed also in the latter reactions.

4. Method B

Method B consists of performing the standard Kahn test as usual with the exception that, after the three-minute shaking period, sodium chloride solution of 2.5 per cent concentration instead of 0.9 per cent is added to the tubes in the regular amounts of 1.0, 0.5, and 0.5 c.c., respectively. When this method is applied to doubtful serodiagnostic reactions, it is frequently observed that in nonluetic cases the results are weaker than those given by the standard Kahn test, while in luetic cases the results are the same or stronger.

5. Combined Use of the Several Verification Techniques in Practice

When a blood specimen reaches this laboratory for a verification test, the serum is separated from the clot in the usual way, checked for clarity, heated for thirty minutes at 56° C., rechecked for clarity, and within about ten minutes after the heating period, the following preliminary tests are performed:

1. *The standard Kahn test.*

2. *Method B,* which consists of the standard Kahn test, in which the diluent added to the tubes after the three-minute shaking period (1.0, 0.5 and 0.5 c.c.) consists of 2.5 per cent salt solution instead of 0.9 per cent.

3. *Salt dispersibility technique.* The results of the above two tests are read and recorded, and without waiting fifteen minutes for rereading, 0.15 c.c., 0.1 c.c., and 0.1 c.c. of 30 per cent sodium chloride solution are added respectively to the three tubes of each test, shaken fifteen seconds, placed for five minutes in a 37° C. water bath and for two to three minutes at room temperature, shaken again for about fifteen seconds and the results read and recorded.

The results of the above three tests are taken into consideration before proceeding with the additional techniques.

In the case of weakly positive reactions: If Method B gives weaker results than the standard Kahn test, it is indicative of a false positive reaction; and in such a case, the salt dispersibility technique will lead to dispersion of the precipitates and to negative results. The differential temperature technique is then performed for confirmation and is likely to give a nonluetic type of reaction. If Method B gives results which are the same or stronger than those of the standard test, the precipitates are generally not dispersible and the differential temperature technique is likely to give a luetic type of reaction. The application of the dispersibility technique to completed differential temperature reactions commonly gives additional confirmation of either the luetic or nonluetic types, as the case may be.

In the case of strongly positive reactions: The results of the standard Kahn test and of Method B are equally four plus and the precipitates may or may not be dispersed by the concentrated salt. The triple quantitative technique is then performed, the results read, and the salt dispersibility technique applied to the completed reactions. Typically nonsyphilitic patterns with the triple quantitative technique are generally confirmed by complete dispersion of the precipitates. Inconclusive patterns with the triple quantitative technique which show dispersibility of the precipitates indicate nonsyphilitic type of reactions. Rarely one encounters a syphilitic pattern in which the precipitates become dispersed. It is believed that in such instances (in the absence of clinical evidence of syphilis) the reaction is in all probability of the nonluetic type.

In the case of negative reactions: The differential temperature technique is employed to determine if a reaction is obtained at 1° C., generally given by "carriers" of false positives, or at 37° C., occasionally obtained in highly treated cases of syphilis.*

AN EXPLANATORY STATEMENT TO PHYSICIANS ON VERIFICATION REPORTS

In this laboratory, the following statement on interpretation is submitted to physicians with each verification report:

The verification test is a supplementary procedure to serodiagnostic tests; its function is to "type" positive serologic (precipitation or complement fixation) reactions on the basis of their specificity; in practice, the test is applicable to cases in which physicians suspect false positive reactions with complete lack of clinical indications of syphilis.

1. "*Type of reaction similar to that obtained in lues*" is reported when, according to the verification test, the positive serodiagnostic reaction shows characteristics of a specific reaction. This type of reaction is commonly noted in known cases of syphilis.

2. "*General biologic (nonluetic) type of reaction*" is reported when, according to the verification test, the positive serodiagnostic reaction shows characteristics of a false positive. This type of reaction is commonly noted in nonsyphilitic cases.

(1) *General biologic (nonluetic) type of reaction in seronegative cases.* Certain nonluetic persons, although giving negative reactions with serodiagnostic tests, may give the general biologic type of reaction with the verification test. These persons are looked upon as potential "carriers" of false positive reactions, giving such reactions off and on with serodiagnostic tests and more frequently with supersensitive tests.

(2) *General biologic (nonluetic) type of reaction in treated cases of syphilis.* Persons who have a tendency to give false positive reactions may exhibit the same tendency after they contract syphilis, and the verification test will then demonstrate the presence of both the luetic and general biologic types of reactions. Following therapy, as these persons are approaching seronegativity, they may give the general biologic type only.

3. "*Negative type*" is reported when the results of the verification test are negative. This type of reaction usually accompanies negative serodiagnostic reactions.

4. "*Inconclusive*" is reported when the verification results are atypical.

5. *Exceptions:*

(1) In isolated instances, physicians have claimed nonspecificity for a given positive serodiagnostic reaction in spite of persistent verification findings of the luetic type.

(2) Transient reactions of the luetic type have been observed in a small percentage of seropositive (nonluetic) cases following infection or immunization.

(3) Transient reactions of the general biologic type have been observed in a small percentage of known cases of early syphilis.

*Studies of the verification test with supersensitive antigens will be reported at a later date.

Interval retesting. Whenever verification results do not conform to clinical opinion or whenever there is a question of the diagnosis of early syphilis, it is of importance to repeat the verification test at intervals of weeks instead of days. Interval retesting will establish the serologic trend in questionable cases with far greater dependability than immediate retesting.

SUMMARY

Nonspecific and specific serologic reactions show similar characteristics with modern serodiagnostic (precipitation and complement fixation) tests for syphilis; hence, these tests do not offer a practical means for the detection of false positives in routine serologic practice. Studies carried out in this laboratory on differential characteristics of nonspecific and specific serologic reactions led to the observation that these reactions have different temperature and electrolyte requirements for optimal reactivity. This observation, in turn, led to the development of several procedures capable of determining whether certain serodiagnostic reactions possess characteristics of specific or nonspecific reactions, or of both types of reactions. These procedures (termed verification test) "type" serodiagnostic reactions on the basis of their specificity and are of practical value, especially when applied to the typing of seropositive cases that are free from clinical indications of syphilis. When applying the verification test to such cases, nonluetic type of reactions may be looked upon as being corroborated by clinical opinion, and the diagnosis of "false positive" can then be made with reasonable certainty.

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CLINICAL AND EXPERIMENTAL

THE PRODUCTION OF CHRONIC HYPERTENSION IN DOGS BY PROGRESSIVE LIGATION OF ARTERIES SUPPLYING THE HEAD*

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THE puzzle of the etiologic relationships of high blood pressure has provided the impetus for quite varied and extensive research. The trend of this work has changed through the years, but there have been two conditions under investigation quite constantly; namely, arteriosclerosis and kidney disease.

While arteriosclerosis is commonly found with chronic hypertension, it is not invariably demonstrable. Furthermore, there may be quite extensive sclerosis, especially of the large vessels, without elevation of the blood pressure. Sclerosis of arterioles has been stated to be quite generally present in chronic hypertension. Gull and Sutton[1] believed this to be the essential pathologic change, alterations of kidneys or other structures being considered as secondary to it. Several studies on the distribution of arteriosclerosis have been made on autopsy material with varied interpretations of the significance of the findings.[2, 3, 4]

Clinical investigation has been carried out by sections of specimens from the skeletal muscles,[5] from the standpoint of correlating sclerosis of arterioles with hypertension. Study of the retinal vessels is easily carried out, and often the changes found are not only diagnostic of hypertension but may indicate definitely the type of hypertension.[6] Such changes may be found to indicate a former hypertension even though the pressure at the time of examination is found to be lowered.[7]

Pathologists have long noted, at autopsies of individuals with hypertension that gross evidence of arteriosclerosis often is marked in one organ and absent or only slight in other organs. Sclerosis of the aorta or its large branches is not necessarily associated with organ changes. The three important structures involved frequently are the kidneys, brain, and heart. In explanation of this association it was usually said that these were "vital" organs and that arteriosclerosis in any such organ was accompanied by increased blood pressure, in order to drive sufficient blood through it to maintain life.

This simple mechanistic explanation is disputed by the work of Page[8] and his co-workers, who interpret their results as indicating the formation of the specific substance "renin" by a damaged kidney, the renin then being changed by a blood activator into the effective antiotonin, which increases blood pres-

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sure. As a result of his extensive and important work on hypertension, Goldblatt,[9] also, believes that the initiating principle derives from the kidneys exclusively.

Only one short note[10] has been found concerning the production of chronic hypertension by operative reduction of blood flow to the brain. Observations of the acute rise of blood pressure with sudden decrease of the cerebral blood supply[11] have long been on record. The injection of kaolin into the cisterna magna has been found to result in hypertension.[12] This is believed to be associated with a communicating type of hydrocephalus, with impaired drainage of the cerebrospinal fluid through the perineural and perivascular lymphatics, although in rats the fluid content of the brain tissue was not increased.[13]

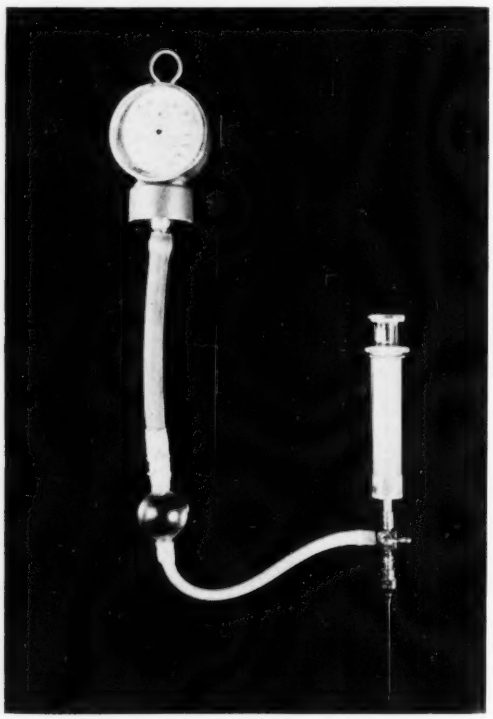

Fig. 1.

We have been interested, over a period of four years, in the effect on the blood pressure of a series of arterial ligations in dogs planned to lessen the blood supply of the brain. The ligations were done in stages, the sequence of operations being changed from time to time. The total series included the ligation of both common carotids in the region of the sixth cervical vertebra, and their dissection upward including the dissection, ligation, and excision of all branches as far out as possible from the bifurcation. Both carotid sinuses were completely removed. Also, the vertebral arteries were ligated just distal to their points of origin, and in the suboccipital region just before they enter the vertebral canal. Afterwards, various exploratory operations were performed for the ligation of any anomalous or collateral arteries which could be found.

As might be expected, the mortality in such a long series of operations was quite high, but decreased with knowledge of when and how to do the ligations. A total of 47 dogs has been used, with 10 of them successfully carried through the series.

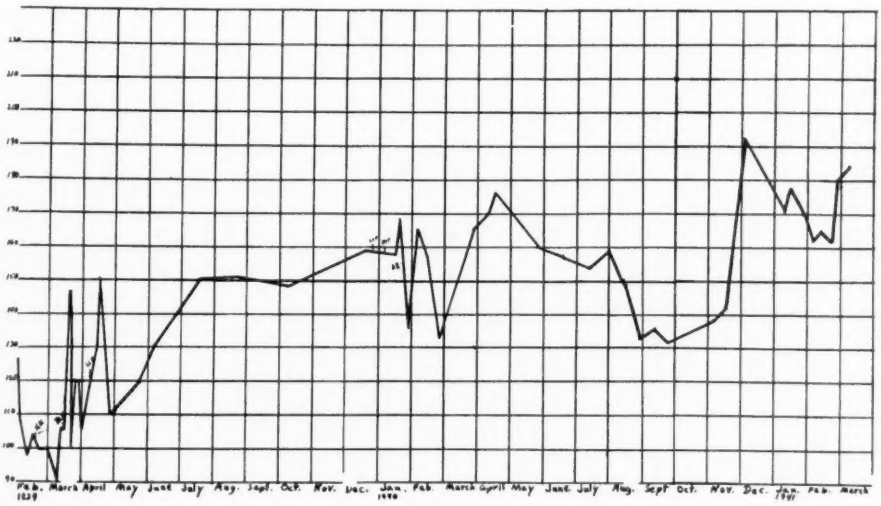
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Fig. 2.—Dog 1.

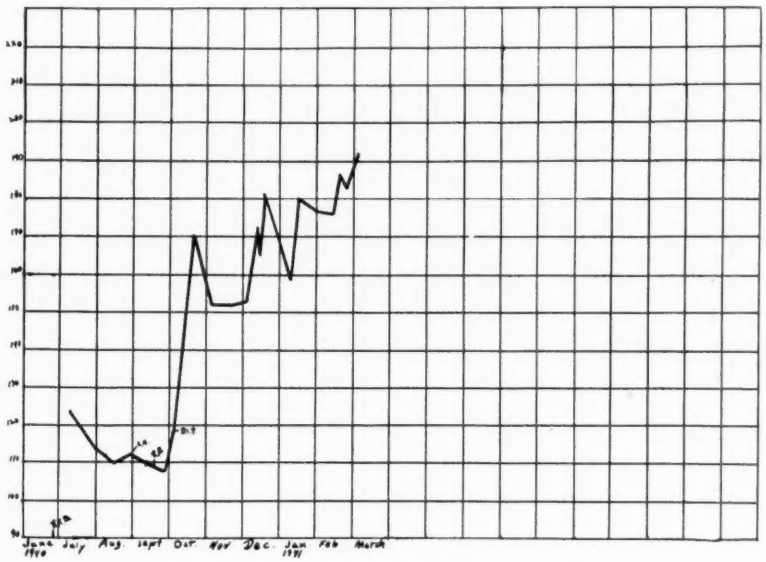

Fig. 3.—Dog 2.

Blood pressures were taken by direct puncture of the femoral artery with a twenty-gauge needle connected through a three-way valve with a glass syringe and an aneroid manometer (Fig. 1). Five per cent sodium citrate solution was drawn up into the syringe and forced out into the rubber tube, so as to fill the glass bulb about one-third full and raise the manometer pressure to about the expected blood pressure of the animal. With the stopcock

turned so as to allow blood to show in the citrate of the syringe, the needle was inserted into the femoral artery and the stopcock then turned to connect the needle with the pressure system. The manometer pointer oscillated from four to six points on the average, and the midpoint of the oscillations was taken as

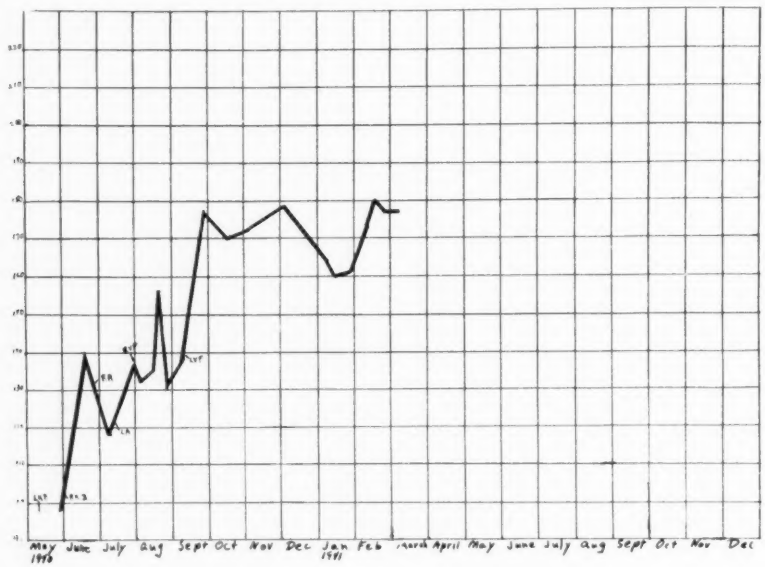

Fig. 4.—Dog 4.

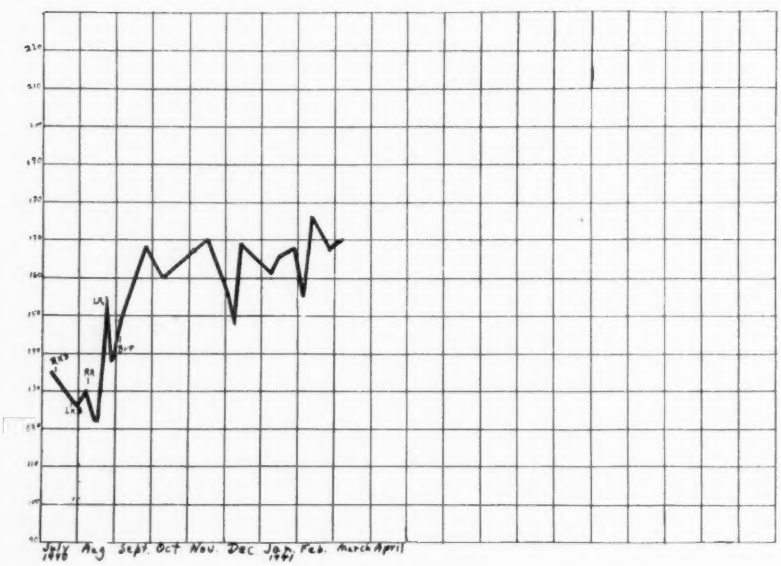

Fig. 5.—Dog 5.

the mean blood pressure. With a period of training the dogs became accustomed to the puncture and would lie quietly while it was carried out. Large dogs, usually of the police dog breed, were used.

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The type of blood pressure response to the ligations, together with the time relationships, are shown in the accompanying graphs. Eight of the ten animals carried through the operative series showed a definite, and some of these a marked, rise of blood pressure. This ranged from controls of 100 to

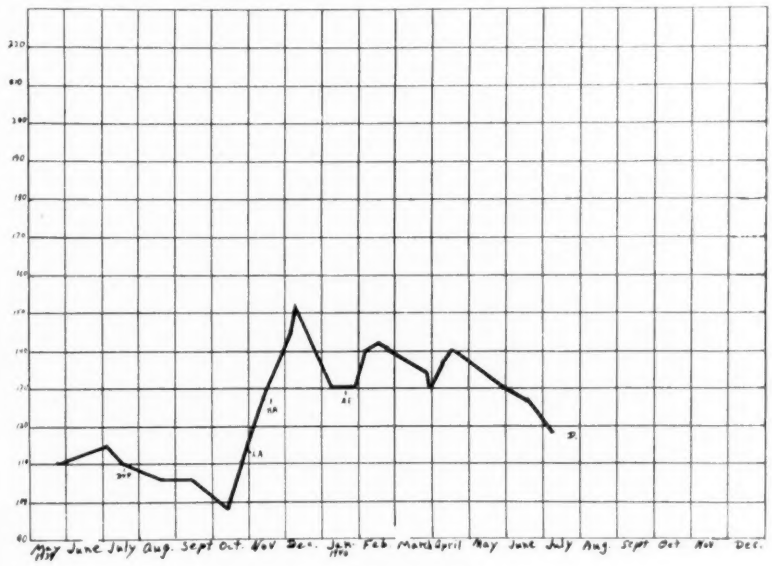

Fig. 6.—Dog 7.

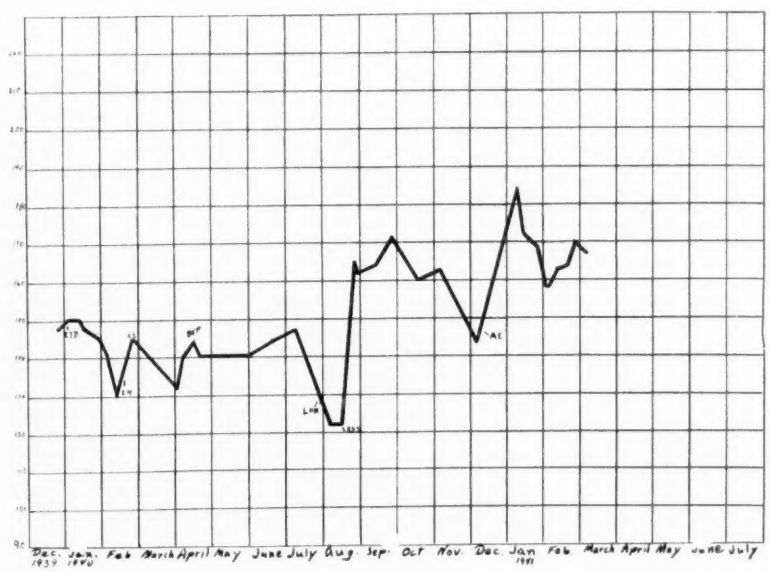

Fig. 7.—Dog 8.

120 mm. Hg usually, up to high points somewhat over 190 mm. Hg. The graphs illustrate the somewhat irregular curves, with variables up to 20 mm. Hg. Such variations occurred from day to day, often for no accountable reason. Two of the ten dogs showed no sustained rise of pressure.

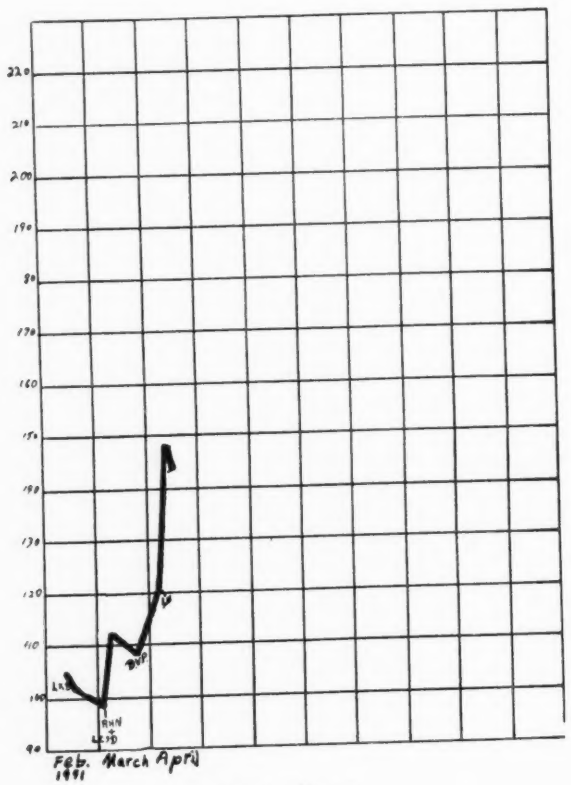

Fig. 8.—Dog 9.

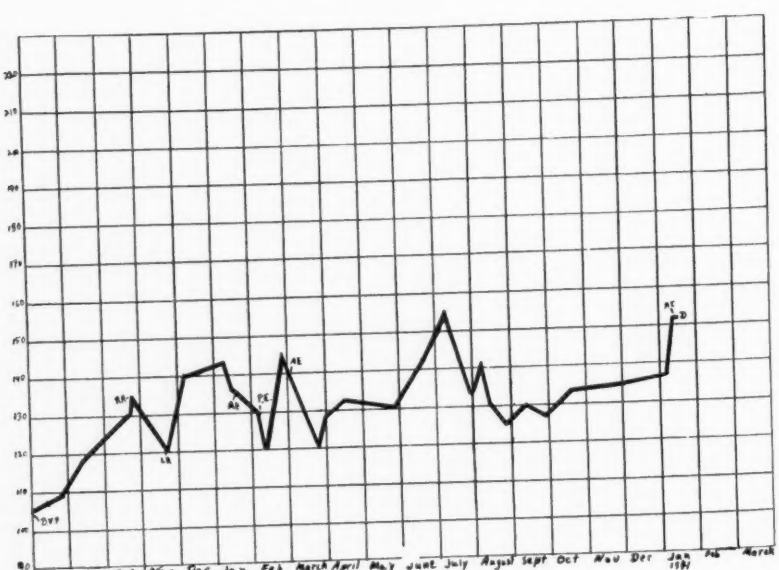

Fig. 9.—Dog 10.

Several workers have reported acute experiments with the production of hypertension by section of the aortic depressor nerves[14, 15, 16, 17] together with denervation of the carotid sinuses. Chronicity of this hypertension has been reported,[18, 19, 20] but others[21, 22] have failed to produce chronic hypertension by this means; this result is shown in our work also.

In four of our ten animals we completely removed the sinuses before the vertebral arteries were sectioned in the occipital region, so that the circulation to the brain was not diminished by their removal. In the graphs of Dogs 2 and 4 the blood pressure is shown to be uninfluenced by removal of the carotid sinuses, whereas hypertension was established later, after the vertebral arteries had been occluded in the occipital region. Experiment on Dog 8 was started by denervation of both carotid sinuses as a single operation. This was done on each side by stripping all nervous and fascial tissue from the common carotid and its branches for a distance of two or more cm. from the bifurcation in all directions. No rise of blood pressure occurred within six weeks. Over a period of five months the series of ligations was performed on this dog, which developed a heightened blood pressure a week after the last operation. Experiment on Dog 9 was begun with removal of the right carotid sinus, complete denervation of the left one, and ligation of the left cranial thyroid artery. No rise of blood pressure occurred within two weeks. Both vertebrals were then tied in the occipital region. After two more weeks both vertebrals and both common carotids were operated low in the neck, and a pronounced blood pressure increase developed.

We believe that denervation or removal of the carotid sinuses has no sustained effect on the blood pressure.[23]

The rise of blood pressure which occurs after multiple cerebral artery ligations appears to be directly associated with the interference with cerebral circulation.

In the dog, cerebral ischemia is difficult to achieve and still more difficult to maintain. Andreyev[24] has studied the development of cerebral collaterals formed after interruption of the major arterial supply to the head. By injection and x-ray he demonstrated quite strikingly the large number of collaterals promptly formed and the arteries involved in their formation. Multiple exploratory operations on our dogs revealed that newly formed arteries developed with a falling blood pressure curve. We are led to believe that once a high blood pressure has been developed by our method, a sustained drop of pressure in an otherwise healthy dog indicates the formation of collateral channels supplying an adequate amount of blood to the brain.

The mechanism by which hypertension is produced by arterial-ligation ischemia of the brain is not known. Either a nervous mechanism or a humoral one may be active. Anrep and Starling[25] demonstrated that a nervous mechanism can initiate hypertension in acute cerebral ischemia. This hypertension was prevented by section of the cord between the vasomotor centers and the sympathetic outflow. The humoral factor had been ruled out.

Page and his co-workers have brought forward evidence to indicate that constriction of the renal artery causes the kidney to produce a humoral mediator of hypertension.[8] Similarly, the hypertension produced by cerebral artery ligation may result from a hypertensive substance formed in the ischemic

brain. To date no work bearing on the presence or identity of such a substance has been performed.

SUMMARY

Chronic hypertension has been produced in dogs by ligation, in series, of the arteries supplying the head.

Removal or manipulation in situ of the carotid sinuses alone has no prolonged effect on the blood pressure of dogs.

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THE HUMAN SKIN AS A CONDUCTOR OF 60-CYCLE ALTERNATING CURRENT OF HIGH INTENSITY, STUDIED ON "ELECTROSHOCK" PATIENTS*

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THE human skin as a conductor of alternating currents has been the subject of many studies,[1] and it was soon shown that it does not act like any ordinary conductor, either metallic or electrolytic. These studies, however, were limited to currents of ten to fifteen milliamperes or less, partly because of the pain produced and partly from fear of causing lasting damage. While currents of higher intensity have been used on the skin of corpses, these experiments were unsatisfactory because of the probability that living and dead skins behave differently. Some information has come from industrial and domestic accidents, but it is far from precise and is frequently vitiated by erroneous computations.[2]

A method has been recently developed of treating mental patients with strong electric shocks applied directly to the head, so as to produce a minor or major convulsion.[3] This has provided an opportunity for extending these experiments and making more precise measurements.

METHODS

The apparatus used for treatment is very simple, consisting of a mechanical relay to make and break the circuit, the timing of which is controlled by a condenser.[4] Round copper plates, 5 cm., serve as electrodes and are attached to the head with even pressure, after a small amount of electrode jelly (sodium chloride, glycerine, tragacanth, and water) has been rubbed into the skin. In some experiments the jelly was omitted. In the first series of experiments the skin resistance of 31 patients was observed during 98 single treatments. Before the "shock" current was passed, a potential difference of about 20 microvolts was applied through the electrodes, and the resulting current was recorded with an ink-writing oscillograph. The impedance, that is, the potential difference divided by the current, was easily determined from a calibration chart.

The impedance-testing circuit was then disconnected, and a voltage varying from 50 to 120 volts was applied through the same electrodes for intervals of $\frac{1}{10}$ to $\frac{4}{10}$ of a second. The "shock" current was recorded by another ink-writing oscillograph during the time of current flow. The ink-writing oscillographs were adapted from the Grass electroencephalograph by using only the final power amplifier for each section.

After the relay had broken the current, twenty microvolts were again applied to the electrodes as quickly as a hand-operated switch would allow. The impedance was then recorded for various times, sometimes extending over 15 minutes. In a second series of experiments, the "shock" current was recorded by a Matthews oscillograph capable of following frequencies up to 15,000 cycles. This was done in order to see whether the form of the current at any time deviates from the sine form.

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RESULTS

In agreement with previous workers who made observations with ballistic instruments,[5] we find that the current resulting from the application of voltages between 50 and 120 is much higher than expected from the impedance originally found. Almost immediately after the current is made, the impedance falls and remains at a low level during the entire time of current flow.

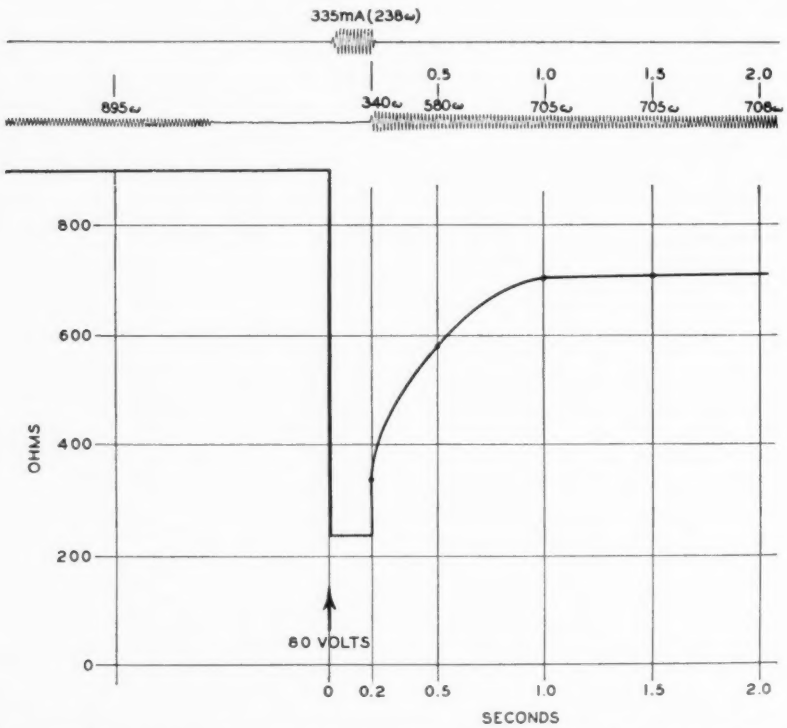

Fig. 1.—Skin resistance to 80 volts A.C. *Upper part:* Original tracings of skin resistance to 20 microvolts before and after "shock" and tracing of current during "shock." *Lower part:* resistance curve as derived from these tracings.

If the initial impedance is 2,000 ohms or less, it drops to a value between 300 and 120 ohms (Fig. 1). The exact value depends on how many volts are given, the lower values occurring after higher voltages. When the current is broken, the impedance rises again. Frequently there is an initial sudden rise of the impedance of 80 to 150 ohms, but in other cases the rise takes place gradually. It then increases slowly over a period of many seconds, and finally approaches the original value asymptotically. After lower tensions the pre-"shock" impedance returns within a shorter interval of time than after higher ones. A second shock, given before the impedance has reached the original value, produces the same current as the previous one; that is, no further drop of the impedance occurs. If all other components are the same, the drop occurs almost instantaneously, and during the next 2 to 3 cycles only a slight further decrease takes place (Fig. 2*b*). If, however, the resistance is higher than 2,000 ohms to begin with, as for example when no electrode jelly is being used, the

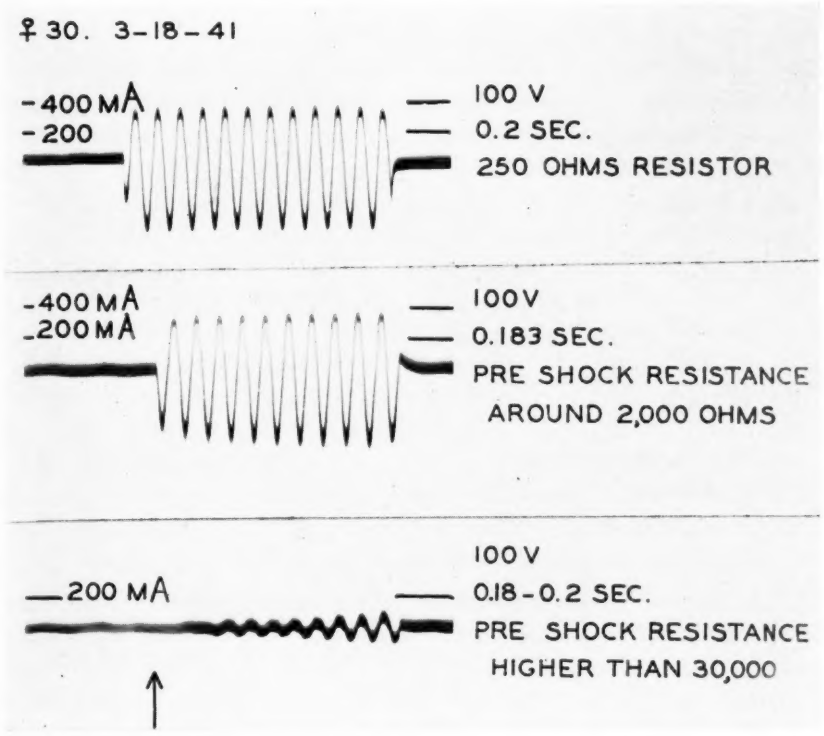

Fig. 2.—Oscillograms (Matthews' oscillograph) of 100 volts A.C. (*a*) passing through 250 ohm wire-wound resistance; (*b*, *c*) passing through human skin.

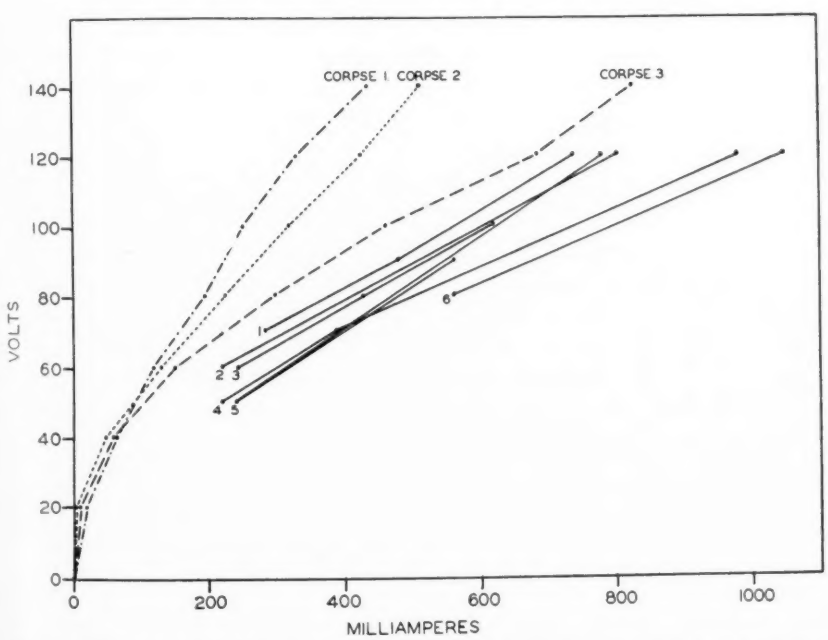

Fig. 3.—Voltage/current for 6 patients and for 3 corpses. Corpse 1 had been dead for 90 hours; corpse 2, for 24 hours; corpse 3, for 3 hours. The drop of the skin resistance when higher tensions are applied is larger in the living skin than in the dying skin. The voltage/current curve of the latter tends to approach a straight line.

current increases very gradually, and assuming the same time of current flow, it may never reach the same high level as otherwise (Fig. 2*c*).

No deviation from the sine form was observed in these experiments, and a few recordings with a cathode ray oscillograph point in the same direction.

The living skin then behaves like an electrolytic condenser which breaks down when tensions above 50 volts are applied. The impedance of the uninjured dying skin of the corpse is of the same order of magnitude as that of living skin for tensions up to 50 volts. If higher tensions are applied, the impedance of dying skin does not drop to such low values as are observed under living conditions. The longer the time after death, the less the drop, until finally the voltage/current curve approaches a straight line (Fig. 3).

Thus, it seems obvious that the sometimes elaborate attempts to measure the resistance of the skin before an electric shock treatment are not necessary. With electrode jelly and the electrodes in good contact, the resistance will usually be below 2,000 ohms and, during the time of current flow, will be between 150 and 250 ohms.

SUMMARY

The reactions of the living human skin as a conductor of 60-cycle alternating current up to 1,000 milliamperes was studied on 31 mentally ill individuals in 98 experiments. When 50-120 volts are given for 0.1-0.4 sec., the impedance drops instantaneously to a value between 270 and 120 ohms regardless of the original impedance, provided this is kept at or below 2,000 ohms. Under these circumstances there is no further change of the impedance during the time of current flow. When the current is broken, the impedance—to 20 microvolts—rises suddenly 80-150 ohms, depending on the "shock" tension applied; it then increases over a period of many seconds, approaching the original value asymptotically. The skin of human corpses reacts similarly to that of living individuals, but the impedance drop is less the longer the interval between the time of death and of examination.

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SOME NEW ASPECTS OF MORPHINE ACTION: INFLUENCE OF PROSTIGMINE METHYLSULFATE ON EXCRETION*

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IT IS now well known (Gross and Thompson[1]) that the greater part of an injected dose of morphine sulfate can be recovered from the urine and feces of accustomed and nonaccustomed dogs. These authors pointed out, however, that a large part of excreted morphine was bound or conjugated and had to be hydrolyzed before it could be recovered.

Further, there is evidence[2] which indicates that prostigmine methylsulfate alters the accustoming† (tolerance-inducing) properties of morphine in dogs.

Recent work[3] suggests that prostigmine methylsulfate enhances the analgetic effects of morphine in cats. If this is true, the reason for the enhancement may lie in the action of prostigmine methylsulfate on the conjugation, destruction, and excretion of morphine.

For these reasons it seemed advisable to determine if the excretion of morphine was influenced by prostigmine methylsulfate. We were particularly interested in ascertaining whether prostigmine methylsulfate altered morphine excretion during withdrawal of morphine, comparatively, in accustomed and nonaccustomed animals and to note if the amounts of free or combined morphine were materially changed. In order to test such a hypothesis, both accustomed and nonaccustomed animals were used.

TECHNIQUE

The animals used in this study were all healthy female dogs. They varied in weight from 8 to 17.7 kg., but animals of similar weights were paired so that the difference in a pair (one accustomed and one nonaccustomed animal) was never greater than 2 kg. Five accustomed and five nonaccustomed animals were studied. A routine as outlined by Pierce and Plant[4] was followed in maintaining the dogs on a constant water and caloric intake. During a 6- to 7-day control period and during a 6 day excretion period, the normal body weights of these animals were not materially altered. The accustomed animals had previously been receiving 20 mg./kg. of morphine sulfate daily for 10 months or longer.

As mentioned above, one point of interest in this study was to compare the level of morphine excretion during withdrawal in accustomed and nonaccustomed

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†We prefer the term "accustomed" to the usual term "addicted," because in our opinion it reflects better the meaning of the French word "accoutumance."

dogs and to note what influence, if any, prostigmine methylsulfate had on the amount and form of morphine excreted. In order to adequately accomplish this, the following procedure was used: each animal, whether accustomed or non-accustomed, received subcutaneous doses of 20 mg./kg. of morphine sulfate daily for an arbitrary three-day period. Then each animal was withdrawn for three days following the last dose. The morphine excretion over this period of six days constituted the control values. With these values were compared the amounts of morphine excreted after the same subcutaneous dose of morphine and over the same period but with the addition of prostigmine methylsulfate in subcutaneous doses of 0.2 mg./kg. administered in conjunction with the morphine as well as during the withdrawal period. The 24-hour output of feces was collected once daily. The animals were catheterized twice daily, and the urines were pooled, comprising the 24-hour output. The 24-hour sample of urine was analyzed individually for free and combined morphine, and the 24-hour sample of feces was analyzed for total morphine.

The extraction of free and of combined morphine after hydrolysis in the urine and the analysis of total fecal morphine was carried out as described by Gross and Thompson.[1] The morphine was determined colorimetrically by the diazotization procedure originally outlined by Pierce and Plant.[4] Known amounts of morphine were added to urine and feces. We were easily able to detect such known amounts and thus establish the validity of the method employed. Control diazotization experiments showed that prostigmine methylsulfate did not in any way alter the determination of morphine by this method.

RESULTS

Table I summarizes the effects of prostigmine methylsulfate on morphine excretion in accustomed and nonaccustomed animals. It is obvious that the total per cent of injected morphine excreted in urine and feces is reduced when prostigmine methylsulfate is administered with the morphine sulfate. This total difference is about the same in both groups of animals although there is a tendency for a greater disparity in the accustomed dogs. If the lessened excretion of morphine is broken down into component parts, the decreased urinary excretion in the nonaccustomed dogs is reflected almost two to one in the combined, as compared with the free form as shown in the following analysis.

ANALYSIS OF TABLE I. URINARY MORPHINE OUTPUT

Under prostigmine methylsulfate the total percentage reduction in the urinary morphine excretion in nonaccustomed dogs (NA) was 17.6 per cent; in accustomed dogs (A), 17.2 per cent; i.e., $\frac{67.2 - 55.4}{67.2} \times 100 = 17.6$ per cent and $\frac{69.1 - 57.2}{69.1} \times 100 = 17.2$ per cent.

From Table I we see that the actual percentage reduction of urinary morphine output under prostigmine is: For NA dogs, *free* 4.2 per cent; *comb.* 7.6 per cent; for A dogs, *free* 5.0 per cent; *comb.* 7.0 per cent. (Since fecal excretion represents only a small percentage of total excreted morphine, it is not considered in this analysis.)

The difference between the reduction in free and the reduction in combined morphine outputs was greater in NA dogs than in A dogs. Expressed as per cent of the reduction in free form for NA dogs, this difference was 81 per cent of the reduction of free form, while for A dogs it was 40 per cent of the reduction in free form; i.e., $\frac{7.6 - 4.2}{4.2} \times 100 = 81$ per cent, etc.

The percentage reduction of excreted morphine after prostigmine methylsulfate was for *Free Morphine* in NA dogs, 28 per cent; in A dogs, 34 per cent; for *Combined Morphine* in NA dogs, 14.6 per cent; in A dogs, 12.8 per cent; i.e., $\frac{15.0 - 10.8}{15.0} \times 100 = 28$ per cent, etc.

TABLE I

EFFECT OF PROSTIGMINE METHYLSULFATE ON MORPHINE EXCRETION IN NONACCUSTOMED (NA) AND ACCUSTOMED (A) DOGS

(Values Represent Average Per Cent Recovery of Total Dose of Morphine Administered)
(All Animals Received 20 Mg./Kg./Day of Morphine for 3 Days)

NUMBER OF ANIMALS		PROSTIGMINE METHYLSULFATE MG./KG./DAY		TOTAL URINE + FECES %		URINE						FECES %	
						TOTAL %		FREE %		COMBINED %			
NA	A	NA	A	NA	A	NA	A	NA	A	NA	A	NA	A
5	5	0.0	0.0	71.8	75.8	67.2	69.1	15.0	14.7	52.2	54.5	4.6	6.6
5	5	0.2	0.2	61.1	62.4	55.4	57.2	10.8	9.7	44.6	47.5	5.6	5.2

TABLE IA

OUTPUT OF FREE AND COMBINED MORPHINE IN TERMS OF TOTAL URINARY OUTPUT AS 100 PER CENT

"PROSTIGMINE"	TOTAL %		FREE %		COMBINED %	
	NA	A	NA	A	NA	A
0.0	100	100	21.3	21.2	77.7	78.8
0.2	100	100	19.5	17.0	80.5	83.0

The percentage reduction in amount of excreted, free morphine is slightly greater in A dogs than in NA dogs but is probably not significant. The percentage reduction in amount of excreted, *combined* morphine is slightly less in A dogs than in NA dogs, but the difference is also probably not significant.

If the figures are expressed as fractions of total urinary morphine found, instead of fractions of the administered morphine, the urinary figures of Table I are as follows: (See Table IA.)

Relative changes: Free morphine output reduction by prostigmine methylsulfate in NA vs. A dogs: NA dogs, 8.5 per cent; A dogs, 19.8 per cent. Hence on a basis proportional to the total morphine found, relative reduction of free morphine output by prostigmine methylsulfate is 2 to 3 times as great in A dogs as in NA dogs after prostigmine methylsulfate. There appears to be a relative *increase* of output of *combined morphine* after prostigmine methylsulfate: in NA dogs, 3.6 per cent; in A dogs, 5.3 per cent.

By way of explanation, the thought arises that perhaps prostigmine methylsulfate has less action on the excretory mechanism than it has on an earlier stage of morphine action and metabolism. That is, that the proportion of free

and combined morphine is an independent factor as far as prostigmine methylsulfate is concerned, and that any variation is more a function of the total amount excreted rather than total dose administered. When total excreted morphine of either accustomed or nonaccustomed animals is considered, we see that after prostigmine methylsulfate, 17 per cent less is excreted.

DISCUSSION

These results clearly indicate that prostigmine methylsulfate decreases the excretion of morphine in accustomed and nonaccustomed dogs. It seems fair to assume that such an effect demonstrates the ability of prostigmine methylsulfate to alter the potential effectiveness of morphine in the animal body. We have long felt that prostigmine methylsulfate prevented the development of tolerance in animals,[2] and clinical observations[5] using combinations of morphine and prostigmine methylsulfate suggest a similar effect.

The greatest actual decrease in excretion of morphine following prostigmine methylsulfate administration is reflected in the combined form. There is also, however, a reduction of output of the free form. In either case it seems possible to suggest that prostigmine methylsulfate alters the effectiveness of morphine in the body, either by causing retention of both forms or by increasing the rate of destruction of both forms in the body so that less can appear in the urine. In proportion to that amount of each form excreted without prostigmine methylsulfate, the greater proportional decrease of output is in the free morphine output; this is also evident in the relative shift in proportion of free and combined urinary morphine shown in Table IA.

Gross[6] has suggested that the combined form of morphine may be responsible for hypnosis and analgesia. If this is true and if retention of both forms of morphine in the body is the mechanism of prostigmine methylsulfate reduction of urinary output of morphine, one might thus explain, at least in part, the potentiation of morphine hypnosis and analgesia by prostigmine methylsulfate. The retention of free morphine might lead to a greater formation of combined morphine, which would still further potentiate the hypnotic action under Gross's hypothesis. Retention would result in a higher concentration of the effective form reaching the loci of action, and thus would simulate the effects of higher doses of morphine.

SUMMARY

1. Prostigmine methylsulfate in suitable doses decreases the total urinary excretion of morphine in accustomed and nonaccustomed dogs.

2. The bulk of the decreased morphine excretion is reflected in the combined form.

3. There is a relative shift in proportions of free and combined morphine in the urine, in the direction of a higher proportion of the combined form. This shift is greater in nonaccustomed dogs than in accustomed dogs.

4. In the light of a retention of morphine in the body produced by prostigmine methylsulfate, the mechanism of potentiation of morphine hypnosis and analgesia by prostigmine methylsulfate is discussed.

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IMMUNOLOGIC AND TOXIC PROPERTIES OF CASEIN DIGEST AS PREPARED FOR PARENTERAL ADMINISTRATION*

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ALTHOUGH hypovitaminosis continues to receive preponderant emphasis in studies on malnutrition, the effects of protein deficiency are not to be minimized. In addition to such obvious effects as inanition, failure of growth, anemia and nutritional edema, protein deficiency (hypoproteinemia) affects both quantitatively and qualitatively the regeneration of tissue; it predisposes the liver to injury by toxins, and it seriously interferes with the synthesis of antibody globulin. Hypoproteinemia is an important cause of delayed wound healing and wound dehiscence.[1] An increased susceptibility of the liver to injury by such toxic substances as chloroform and arsphenamine during a state of protein depletion has been shown, and the fact that feeding of protein exerts a marked protective action against injury has been demonstrated.[2] The effect of protein depletion on resistance to infectious disease was the subject of discussion in the presidential address to the American Association of Immunologists this year.[3] Evidence was presented to show that hypoproteinemia in animals markedly decreased their ability to synthesize antibody globulin, thus interfering with one of their most important defense mechanisms against infectious disease —the ability to develop active immunity.

The treatment or prevention of the protein deficient state is often difficult. Administration of protein by mouth may be impossible, or digestive disturbances may prevent assimilation of fed protein. Associated with these conditions there is often a marked increase in protein requirements either from abnormal loss of protein or from increased metabolism. Parenteral therapy to relieve a condition of protein deficiency would be of especial importance in preparation of patients for surgery as well as in helping to maintain their nutritional state during the early postoperative period. In a discussion of hypoproteinemia and its relation to surgical problems, Ravdin[4] states, "No consideration of fluid and electrolyte loss and their restitution is sufficient unless the plasma protein is simultaneously considered."

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For these reasons a source of protein which may be safely administered parenterally is much to be desired, to supplement or complement solutions of carbohydrates, salts, and vitamins.

Over thirty years ago experiments were first done to develop such a source of protein. Success has come only recently, however. The gradual accumulation of facts leading to the demonstration that protein requirements could be completely or almost completely met by supplying ten "essential" amino acids was probably the most influential factor in this respect. In a Harvey lecture in 1934[5] Rose said, "Indeed it is not wholly improbable that methods may be developed whereby this ideal mixture (amino acids) may be employed for parenteral administration to human subjects when the prevention of undue loss of body structure is an important consideration."

This "ideal mixture" of amino acids has been closely approached in recent years although it comes not from a synthetic mixture of purified amino acids as Rose had postulated, but from the hydrolysis of a complete protein. Rapidly accumulating experimental data[6, 7, 8, 11, 13] indicate that such preparations are utilized for protein synthesis and that when given parenterally, as a sole source of nitrogen, they serve to maintain nitrogen equilibrium and support normal growth and tissue repair.

A commercial preparation of casein hydrolysate designed to serve as a parenteral source of protein food has been available for experimental use and has received much study during the past five years.* Experience with new drugs (e.g. amidopyrine, dinitrophenol, sulfanilamide, etc.), has indicated, however, that although initial experimental trials reveal no significant toxic action, there may be decided deleterious effects when such drugs are subject to widespread or general use. For this reason, new drugs should always be subjected to thorough experimental study expressly designed to detect or to determine the nature of possible toxic effects. The demonstration of such toxic or deleterious action of specific drugs need not result in absolute contradiction to their use; yet the knowledge of such untoward effects may allow for intelligent, directed caution or for the application of corrective measures. With these thoughts in mind experiments were undertaken to determine whether or not amino acid solutions prepared from hydrolyzed casein were toxic.

In preparations made from proteins and intended for intravenous administration to human beings the possibility of allergic reaction must always be considered. Since casein is an antigenic protein, there is the possibility that a product derived from casein might retain some of these antigenic properties. The first consideration, therefore, is to determine whether or not casein hydrolysate is antigenic. Since it is generally accepted that complete hydrolysis destroys the biologic speciation of native proteins, demonstration of nonantigenicity in these products would be strong evidence that complete hydrolysis had been accomplished.

Two commercial preparations of casein hydrolysate were selected for testing. These will be referred to as products A and B. Concerning product A, its manufacturer states that efforts have been made to sensitize guinea pigs with A and to shock milk-sensitive pigs with A solution and that all such studies have been negative, indicating the absence of antigenic properties; however, no

*Amigen, an enzymatic hydrolysate of casein, prepared by Mead Johnson & Co.

details of these experiments are available in the literature, nor are there experiments specifically directed toward the detection of pork protein which might have been introduced during the course of hydrolysis, a process accomplished by the action of ground pork pancreas. Concerning the antigenic properties of product B, an acid hydrolysate of casein, its manufacturer states that "biologic speciation in most instances is due to the prosthetic groups attached to the protein. Most proteins shown to be antigenic substances are despeciated by enzymatic hydrolysis." The product in question, however, is not an enzymatic hydrolysate. The works of Hill and of Altshuler are cited to substantiate further claims that this particular casein digest is nonantigenic. Neither Hill nor Altshuler used the particular preparation under consideration. Hill[7] maintained infants allergic to milk on casein digest for periods up to three months. In about half of the cases eczema was relieved. Altshuler, Hensel, and Sahyun[8] injected two rabbits with an acid hydrolysate of casein and were subsequently unable to produce anaphylactic shock in these animals. These findings are inconclusive, however, because of the unsuitability of rabbits for anaphylactic tests.

EXPERIMENTAL

ANTIGENIC PROPERTIES OF CASEIN HYDROLYSATES

The first series of experiments consists of determining whether or not preformed antibodies to casein react with hydrolysates of casein and whether or not hydrolysates of casein are in themselves capable of stimulating the formation of antibodies for themselves or for casein. Three groups of normal adult rabbits were given repeated injections of preparation A,* preparation B, and purified casein (Casein-Harris). Multiple doses of these substances were administered by intramuscular, intraperitoneal, subcutaneous, and intravenous routes. In addition, ten intramuscular "depots" of the test substance adsorbed upon alumina gel after the method of Hektoen and Welker[9] were produced. After 65 days serum precipitin titers were determined in all groups by the collodion particle method of Cannon and Marshall,[10] as well as by the usual antigen-dilution method. Precipitins for casein digest were not found in any case; neither did the animals treated with casein digest exhibit antibodies for casein. Skin tests were performed using cows' milk, 10 per cent A, and 10 per cent B. Casein-sensitized animals gave positive reactions only to milk. Animals treated with casein digests gave no positive reactions to casein nor to the casein digest with which they had been treated. These animals were bled, and the pooled serums of each group were injected intraperitoneally into corresponding groups of normal 180- to 200-gram guinea pigs. Two days later anaphylactic tests were performed. Two c.c. portions of the test substances were given via the jugular vein to the unanesthetized pigs. Casein-sensitized animals were divided into three groups. To group one, casein was given and these animals died of typical anaphylactic shock. Group two received A (10 per cent solution) with no reaction. After several hours these same animals were again injected, this time with casein, with resultant death from typical anaphylactic shock. Group three was treated in essentially the same manner as group two, except that solution B was substituted for A. Identical results were obtained.

*Kindly furnished by Mead Johnson & Co.

Twenty-eight virgin female guinea pigs weighing 150 to 180 grams were "passively sensitized" with pooled serums from rabbits treated with casein, A, or B. These animals were sacrificed at three to four days, and muscle strips (uterine horn) preparations were studied for passive sensitization using the Schultz-Dale technique. Fig. 1 shows the typical reaction of a normal uterine muscle strip to A, to B, and to casein. These test solutions (aqueous) had been adjusted to a pH of 6.8 to 7.0 by the careful addition of 0.1 N NaOH. In Fig. 2, however, the pH of test solutions A and B was unaltered (approximately 4.5). These differences in reaction to A (compare Figs. 1 and 2) are apparently the result of some change in state that occurs with neutralization of solution A, a change that is marked by the gradual formation of a rather abundant cloudy precipitate.

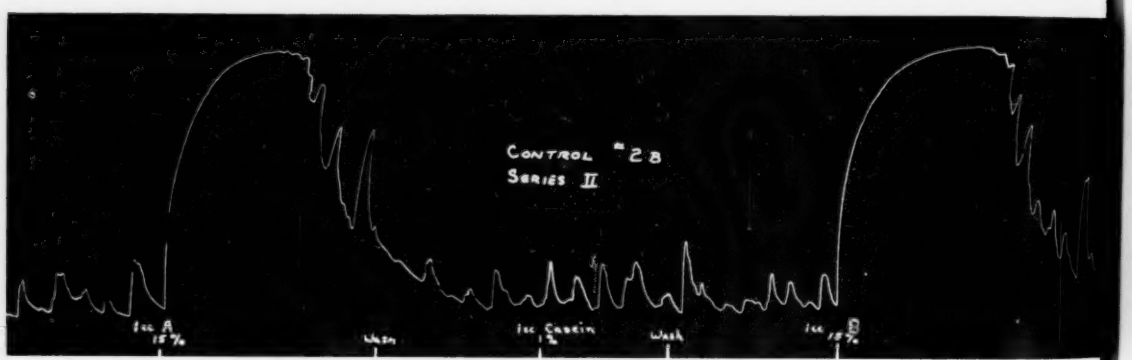

Fig. 1.

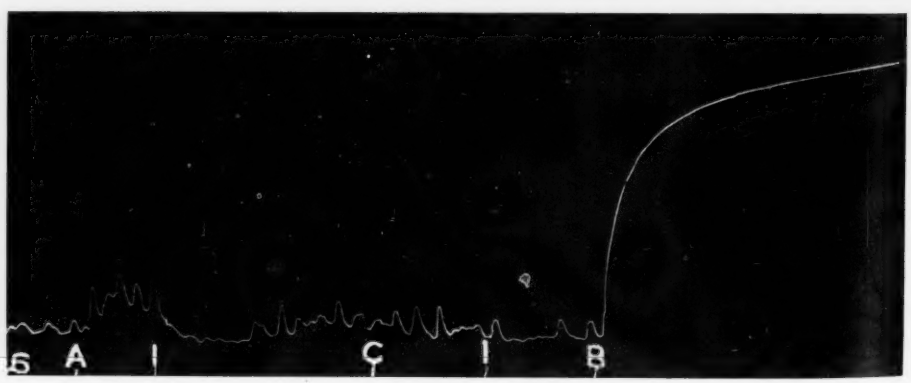

Fig. 2.

The reaction of normal smooth muscle to these hydrolysates of casein indicates the presence of some histamine-like substances, peptones, or tyramine, which may explain the frequently observed reactions of flushing, sensation of warmth, and nausea observed in patients who are administered casein digest by vein.[11, 13]

Typical reactions of the casein-sensitized group are shown in Fig. 3. An anaphylactic response has occurred to casein. The reactions of this group to solutions A and B are essentially similar to those exhibited by the control animals.

Fig. 4*A* shows a typical reaction of the A-"sensitized" group to casein and to A; these are normal reactions. Fig. 4*B* illustrates the reaction of a B-"sensitized" animal to casein and to B. This reaction to B is the most marked of any exhibited by this group; it is not a typical anaphylactic reaction, however, and the reaction to casein is as in the normal. Other animals of this group gave an essentially "normal" reaction to B.

These studies adequately demonstrate that the two preparations of hydrolyzed casein tested are incapable of stimulating antibody production, nor do they react to preformed casein antibodies.

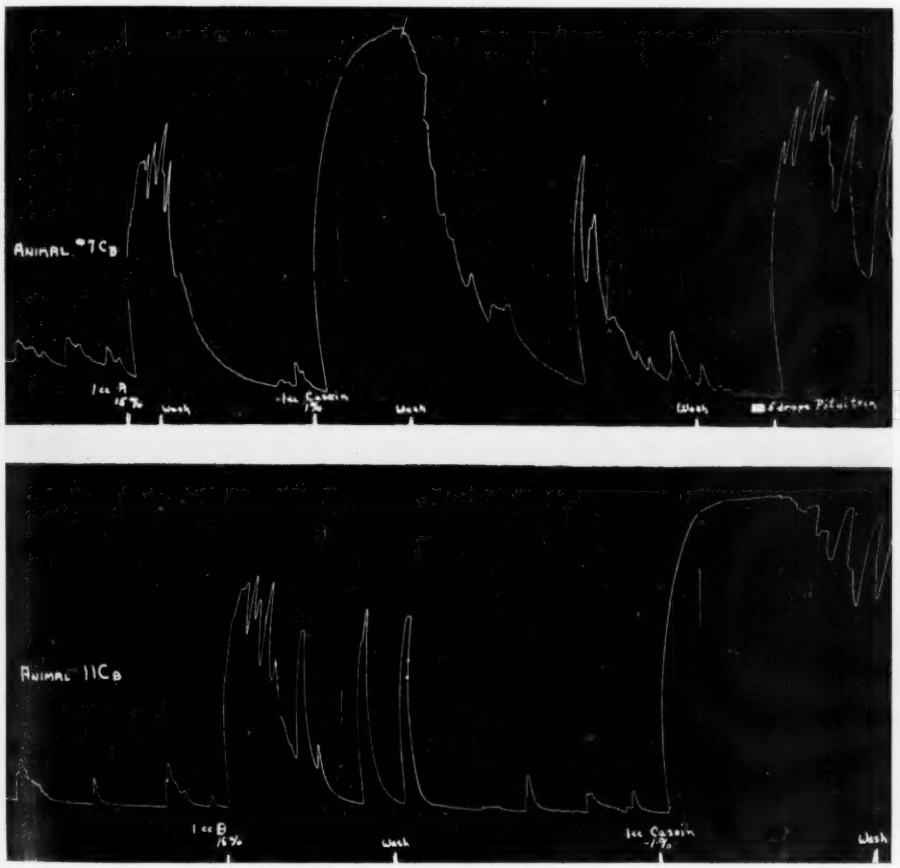

Fig. 3.

A second series of experiments concerns only product A in the preparation of which hydrolysis is accomplished by means of ground pork pancreas. The nonantigenicity of substance A has just been demonstrated, but there remains for investigation the possibility that modified pork protein exists as a partial antigen or haptene, incapable of stimulating antibody formation, but capable of reacting with preformed pork antibodies. To explore this possibility, rabbits were sensitized to swine pancreas and swine serum, as demonstrated by the presence of specific precipitins and by positive Arthus reactions. Animals treated with A failed to show precipitins or positive skin reactions to swine

serum or swine pancreas. Likewise, animals sensitized with swine pancreas or swine serum failed to show precipitins or positive skin tests against A. Anaphylactic tests were performed with passively sensitized guinea pigs in the same manner as previously described. There was no demonstrable antigenic relationship between pork proteins and product A.

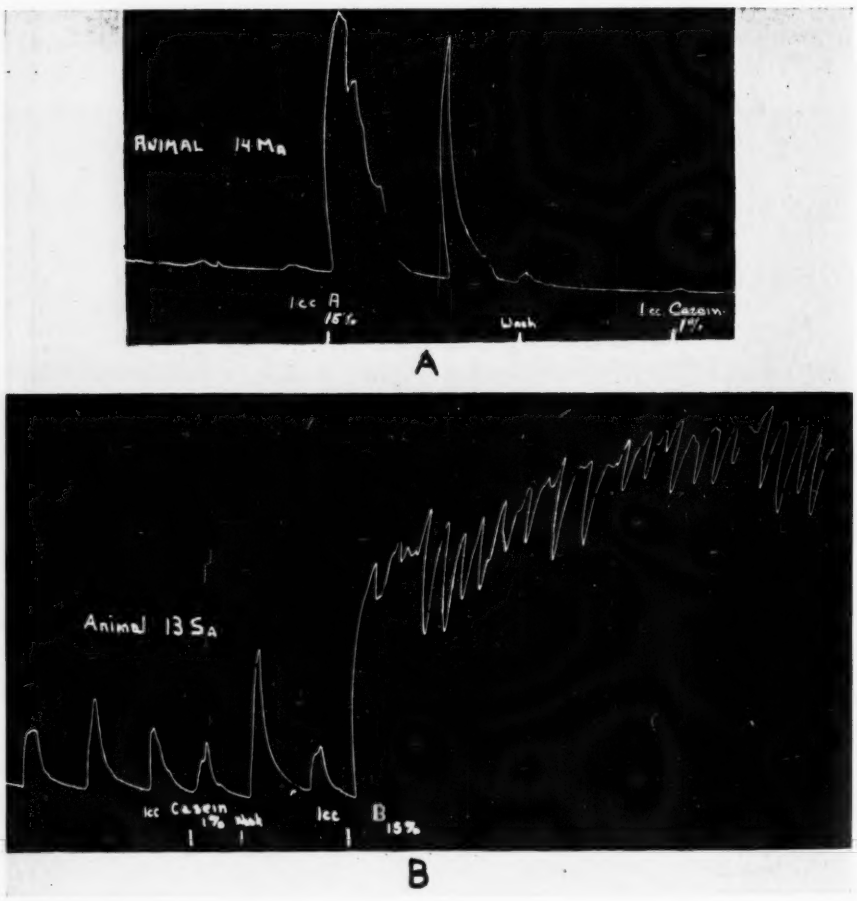

Fig. 4.

TOXIC PROPERTIES OF CASEIN HYDROLYSATES

Table I shows the hydrogen ion activity and hydrogen ion concentration of the two preparations tested. It can be seen that these solutions have a hydrogen ion concentration comparable to 1/16 to 1/12 N HCl. Their effect on the pH and CO_2 combining power of human plasma in vitro is also shown. The proportions of hydrolysate and plasma in these mixtures is comparable to that which would be attained in a 65 kg. patient given intravenously, at a single dose, 1500 c.c. of a 10 per cent solution of amino acid mixture. This is admittedly an artificial comparison in that amino acid mixture introduced into a patient would not all remain in the blood stream for the period required to administer this large quantity. From these observations it was thought that

casein hydrolysates might have a marked tendency to produce acidosis, especially in those patients who were seriously ill and who received repeated large doses of the substance. Experiments were done to evaluate this effect. Blood plasma was obtained from two patients* just before the intravenous administration of 1500 c.c. of 10 per cent amino acids (product A). This material was given over a period of eight hours and was accompanied by 1500 c.c. of 10 per cent dextrose in saline and 1500 c.c. of 10 per cent dextrose in water. Immediately upon completion of this infusion a second specimen of plasma was obtained from each patient. In patient No. 1, the CO_2 combining power dropped from 59.2 vol. per cent in the first sample to 50.5 in the second sample. In patient No. 2, the decrease was from 58.7 vol. per cent to 52.6 vol. per cent. These changes may well represent only the dilution of blood plasma resulting from administration of a large amount of hypertonic fluid. Plasma pH did not vary significantly. Similar observations were made on a third patient† to

TABLE I

HYDROGEN ION ACTIVITY AND CONCENTRATION OF CASEIN HYDROLYSATES AND THEIR EFFECT ON HUMAN PLASMA

SOLUTION	pH	0.1 N.NaOH ADDED TO BRING pH TO: (10 C.C. SAMPLE)	
		7.2—7.4	8.1—8.2
10% A	4.6	5.59 c.c.	12.82 c.c.
10% B	4.33	8.15 c.c.	13.98 c.c.
		CO_2 Combining Power Vol. %	
Plasma + Saline	7.5	60.9	
Plasma + A	5.96	6.9	
Plasma + B	5.51	5.8	

whom 400 c.c. of 10 per cent amino acids was given intravenously during a period of two hours and thirty minutes. Variation in CO_2 combining power and pH of plasma did not exceed the limits of experimental error.

From these in vivo studies it is apparent that hydrolyzed casein, in spite of its very appreciable hydrogen ion activity in vitro, has but little effect on the acid-base balance in the body. Since but a small proportion of this substance, given intravenously, is excreted as such in the urine, and since there is but little immediate destruction of the substance, as measured by increased ammonia excretion, it appears necessary to postulate the very rapid conjugation of parenterally administered amino acids in order to explain how the acidity of such solutions is nullified without affecting the alkaline reserve of the blood. That such conjugation of amino acids results in the immediate formation of complete proteins seems hardly likely although such a step may be a major one in the synthesis of protein. This conclusion is in harmony with direct observation on the rapidity with which the blood stream is cleared of injected amino acids.[12, 13]

To further test for toxic action of casein hydrolysate, post-mortem studies were made on seventeen patients who had received casein digest by intravenous route during the course of their terminal disease. Of these patients, the maximum

*These individuals were both adult women. Patient No. 1 had undergone a cholecystectomy, and patient No. 2, an appendectomy, the preceding day. They both made uneventful recoveries. These patients were on the service of Dr. Alexander Brunschwig.

†A 56-year-old white man suffering from lipoid glomerular-nephritis (nephrotic nephritis).

amount administered was 2,100 Gm., as 10 per cent solution (over a 21-day period). Time of administration of amino acids in these patients varied from twenty days to one hour before death. In no case were there morphologic changes in tissues which could be definitely attributed to a toxic effect of casein hydrolysate. In but one instance was there any reason to suspect that amino acid therapy was a factor in causing death. This was in a man with carcinoma of the cystic bile duct who had undergone an operation in which the gall bladder and cystic duct were resected, the common bile duct ligated, and the hepatic artery ligated and severed. Postoperatively, this patient's course was one of progressive failure. There was jaundice and, two days before death, a precipitous fall in serum protein. In an attempt to ameliorate this condition amino acid therapy was instituted. Following the intravenous administration of 200 c.c. of 10 per cent amino acid solution, the patient experienced a severe chill. The venous infusion was stopped, but the patient died an hour later. At necropsy severe hepatic damage was evident.

That the liver is the major organ concerned with the synthesis of protein seems evident from the work of Whipple, Mann, and others; in fact, the disappearance curve for injected amino acids has been used with some success in evaluating liver function. The study of intermediary protein metabolism with the aid of isotopes by Schoenheimer and others also lends support to the view that amino acids, plasma proteins, and tissues proteins, especially those of the liver, exist in a state of very dynamic equilibrium. In a recent paper,[14] Madden and Whipple summarize such experimental evidence and conclude, "The present evidence speaks in favor of the liver as the site of production of albumin and much of the globulin."

The case last cited may well represent a situation in which hepatic damage was so severe that rapid conjugation of amino acids, as a preliminary step in the synthesis of protein, could not occur, with the result that amino acids given intravenously exerted the same effect on the acid-base balance of the body as was observed in in vitro experiments with human plasma (see Table I). It suggests that marked hepatic insufficiency may be a very serious contraindication to the parenteral administration of amino acid mixtures.

SUMMARY AND CONCLUSIONS

It has been demonstrated that the two preparations of casein hydrolysate tested are nonantigenic. Reactions of normal smooth muscle strips to casein digests have indicated the presence of some histamine-like substances, peptones, or tyramine, which may explain the frequently observed reactions of flushing, sensation of warmth, and nausea, in patients administered casein hydrolysate by vein. It has been shown that although casein hydrolysates have a high hydrogen ion concentration and activity, this does not appreciably affect the acid-base balance in those persons with adequate liver function. This is presumably due to the very rapid rate at which amino acids are conjugated by the liver. Post-mortem studies of seventeen individuals who had received casein digest parenterally failed to disclose significant morphologic changes as a toxic manifestation of amino acid therapy. The suggestion is made that patients with poor liver function may be unable to conjugate parenterally administered amino acids at a sufficiently rapid rate to escape severe acidosis.

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CAFFEINE-WITHDRAWAL HEADACHE*

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THE production of the experimental headache for investigative purposes was initiated by the observations of Loevenhart[2] and his co-workers in 1927, who found that nitrite headaches were accompanied by a dilatation of the meningeal blood vessels. Pickering[3] in 1933 studied headaches produced by the intravenous injection of small amounts of histamine. These studies were enlarged by Clark et al.[4] in 1934 and by Wolff et al.[5] in 1940, who demonstrated that this experimental headache was accompanied by an increased excursion of the cerebral blood vessels. Carbon monoxide headaches have been studied in a few individuals (Cobb et al., 1924),[6] but the toxic nature of carbon monoxide does not lend itself to human experimentation. The experimental headaches studied thus far do not simulate any of the so-called common headaches in mode of onset or duration. The histamine headache, however, is by far the most constant and may be produced in almost all subjects.

Because many people ascribe an occasional headache to lack of their morning coffee, and because considerable "letdown" must result if this stimulant drug is withdrawn from habituated individuals, an attempt to produce and study such a headache was undertaken. In a recent survey of one hundred and twenty-eight migraine patients, twenty-five stated that lack of their usual coffee intake would result in headache. Five patients volunteered the information that the headache was not of the migraine type. In this study we have attempted to produce headache by administration of caffeine over a suitable period, usually a week, and then abruptly withdrawing the drug.

Data obtained in this laboratory[7] have indicated that there is a relative hemoconcentration accompanying migraine. Hence, it was thought advisable to determine total proteins and hematocrits during the course of the experiments. Several investigators[8-11] have reported that the serum calcium is decreased in migraine patients. Therefore, it was thought possible that electrolyte changes in the blood might also be correlated with any period of headache produced by caffeine withdrawal.

EXPERIMENTAL

Caffeine, 0.120 Gm. capsules (gr. ii) as the sodium benzoate or citrate salt, was administered to normal subjects in an increasing daily dosage up to 0.78 Gm. (gr. xii), usually given in the morning. Twenty male and two female subjects (graduate and medical students) were used. Their ages varied from

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20 to 33 years. They were not hospitalized, but they were observed and had their symptoms recorded daily. No regulation of food intake was attempted. Only three of the twenty subjects were normally total abstainers from caffeine-containing beverages. In order to add to our knowledge of the migraine syndrome, five of the subjects were particularly chosen because they had had typical periodic migraine headaches for at least two years. The subjects were given a series of ten or more dated envelopes, each containing the same number of capsules, placebo capsules and caffeine capsules being mixed in such a way that the subject would start with a small dose of caffeine the first day, but increasing daily so that by the sixth or seventh day the subject would be receiving 10 to 12 grains of caffeine per day. On the seventh or eighth day placebo capsules were substituted without the subject's knowledge, so that no caffeine was received thereafter. During the experiment the subject abstained from caffeine beverages, and in most cases the subjects had no caffeine beverages for the 24 to 48 hours preceding the beginning of administration. The experiment was postponed if the subject had a headache, and this occurred in five instances when habituated subjects discontinued their customary daily coffee intake.

Five blood samples were taken during each experiment. An initial control sample was taken before administration of caffeine was started, a second at the height of the caffeine stimulation, a third on the day before withdrawal, a fourth at the height of the headache, and a fifth on the following day when the subject was free of headache. In tabulating the results, the first three samples on each subject have been averaged, since they were found to be uniformly the same. At the time the blood samples were taken, blood pressure and pulse were recorded after the subject had been supine for five minutes. All control observations and samples were taken between 4 P.M. and 8 P.M. after it was found that this was usually the time of maximum headache.

Serum calcium, serum inorganic phosphorus, serum potassium, serum protein, serum and blood specific gravity, and hematocrits were determined as in previous studies.[12] The calcium samples were stored at a stable phase of the analysis, so that fifty to one hundred samples could be disinterestedly titrated.

RESULTS

During the first two or three days of caffeine administration, all subjects reported stimulation with slight muscular tremors and a lessened sense of fatigue. Two subjects noted insomnia immediately upon retiring, but this was never severe enough to require sedation. Later they noted no stimulation, but did report that they slept longer and more soundly. Approximately one-half of the subjects reported diuresis during the early period of the administration. The lack of stimulation on repeated doses agrees with the findings of Eddy and Downs,[13] who noted an increase in the reaction time in habituated subjects.

In twenty-one out of thirty-eight trials, headache resulted which was as severe as the subject had ever had. In eleven trials the headache was definite, but not severe enough to necessitate treatment. In six experiments on four subjects very slight or no headache resulted. The headache-resistant group did not include any of the original abstainers from caffeine. In three of these re-

sistant subjects, the icteric index was above the normal range[23, 11, 8] and the sclerae of M. B. with an index of 23 were yellow-tinged. These three subjects have noted that they are generally free of headache of any type. However, subject M. B. with idiopathic jaundice had a typical histamine headache of 12 minutes' duration when given 0.1 mg. of histamine diphosphate intravenously. Both of the two female subjects developed severe headache which required treatment.

Characteristics of the Headache: The symptomatology of the headache is remarkably constant and may be summarized as follows: On the day of withdrawal, lethargy was usually noticeable in the morning, while a feeling of cerebral fullness occurred about noon. Actual headache usually began in the early afternoon and reached a peak three to six hours later. The subjects localized the headache as occipital or central at the onset, which then in most cases became generalized and throbbing in character. In six subjects the headache was consistently accompanied by some degree of nausea, and in two subjects vomiting occurred. In four subjects the headache was consistently accompanied by a serous rhinorrhea of such severity that they at first thought that an acute upper respiratory infection was impending. Subjects also complained of mental depression, drowsiness, yawning, and disinclination to work.

During the incipient stage of the headache the patients' ears were usually intensely hyperemic, but later if the patients became nauseated, the ears would pale and generalized pilomotor activity was observed. The headache, when well established, was intensified by bending over or by straining, as at stool (Valsalva's experiment). Relaxation, such as napping, made the headache worse, while violent physical exercise (running upstairs), with the exception of one subject, intensified the headache. Jugular vein compression with the resultant increase in cerebrospinal fluid pressure produced the usual feeling of cerebral fullness, but did not intensify the headache.

Treatment of the Headache: Headache produced by caffeine withdrawal was found to respond to treatment by caffeine or acetylsalicylic acid. When caffeine was used to relieve the headache, the subjects sometimes complained of secondary headache the next day. While benzedrine sulfate gave relief for two to six hours, in four of six subjects the headache recurred in an intensified form. Amyl nitrite afforded temporary relief in one subject, but this was then followed by a short period of exacerbation, after which the headache continued as before. In severe headache with nausea and vomiting, oxygen inhalation afforded definite relief in two subjects. If given early enough in the course of the headache, the most effective treatment was caffeine.

Blood Findings: In Table I are given the results of determinations of serum calcium, serum inorganic phosphorus, serum protein, and hematocrit during the normal and administration periods, at the time of the headache, and on the day following when the subjects were free of headache or had received caffeine in treatment of a secondary headache. Under each column are given the averages and standard deviations of all the data. Values obtained during the administration of caffeine are not tabulated separately because these values were comparable to the normal values. The serum protein and the hematocrit were found to be generally lower at the time of the headache in all but three

experiments. The serum calcium was lower in all but one experiment, and serum inorganic phosphorus was slightly higher during the headache period. The change in serum calcium could be ascribed to the hemodilution except that by the same reasoning the rise in serum phosphorus would be even greater than is apparent from the data. Since calcium fluctuates inversely with phosphorus, the calcium may still be indirectly involved.

Graphs of changes in blood constituents in one subject during three different experiments are shown in Figs. 1 to 3. It should be noted that at the time of the headache, significant, but not consistent, decreases occurred in the serum calcium, protein, hematocrit, and blood specific gravity. An elevated serum phosphorus was consistently present at the time of the headache. The blood pressure and pulse changes were frequently marked, but were in general uninterpretable.

TABLE I

SUBJECT		CALCIUM MG. PER 100 C.C.			PHOS. MG. PER 100 C.C.			PROT. GM. PER 100 C.C.			HEMATOCRIT %		
		1	2	3	1	2	3	1	2	3	1	2	3
D	10/7	9.6	9.5	10.0	3.2	4.6	4.2	8.2	7.6	8.3			
P	10/17	9.6	9.2		4.3	4.2	3.0	7.7	8.1	7.6	47	48	49
R	10/27	9.8	9.4	9.5	2.6	3.6	3.0	8.1	7.8	8.3	48	45	48
KA	11/10	9.5	9.2	9.0	3.0	3.2	3.1	8.8	8.5	8.2	49	48	49
RS	11/10	9.0	8.1	8.2	2.7	3.4	3.2	8.0	7.5	7.5	47	46	49
BF	12/9	9.6	9.0	8.1	3.5	3.8	4.1	8.3	8.0	7.9	47	44	46
D	12/1	9.6	9.5	10.0	2.9	3.2	3.4	8.3	8.7	8.9	47	46	51
D	12/29	10.3	9.3	10.9	3.6	3.1	3.4	8.8	7.7	8.8	51	48	49
R	1/19	9.9	9.5	9.8	2.6	2.9	2.7	8.7	8.4	8.7	48	45	47
P	2/23	9.3	8.9	9.5	3.0	3.2	3.6	8.0	7.1	8.7	50	46	48
D	2/23	9.4	9.1	8.9	3.6	3.6	2.8	8.2	7.8	7.6	52	48	50
BF	4/6	9.2	9.0	8.6	3.4	3.3	3.4	7.6	7.4	7.2	43	45	44
DH	4/15	9.9	9.5	9.7	2.8	2.8	3.7	8.1	7.9	8.3	50	49	51
RS	4/23	8.9	8.9	8.9	3.6	3.8	3.0	8.2	7.7	7.8	47	46	43
RS	6/14	9.2	8.5	9.1	4.3	4.7	3.6	8.4	8.4	7.0	48	45	49
DB	5/6	9.6	9.3	10.8	3.5	3.6	3.7	8.0	7.9	9.6	49	48	47
CL	6/29	9.0	8.8	8.8	2.6	3.5	4.1	7.2	7.5	7.4	48	51	47
Average		9.5	9.1	9.4	3.2	3.6	3.4	8.2	7.9	8.1	48.2	46.7	47.9
Median		9.6	9.2	9.3	3.2	3.5	3.4	8.2	7.8	8.2	48	46	48
S.D.		±.4	±.4	±.8	±.5	±.5	±.4	±.4	±.4	±.5	±2	±2	±2

The first column in each series represents the average of all determinations taken before the headache. Column 2 represents determinations at the time of the headache, and column 3 represents determinations 24 hours after the headache. The mean of 12 control calcium samples taken daily at 5 P.M. on 4 subjects for 3 days was 9.45 ± .57 mg. per cent.

DISCUSSION

It was uniformly noted that the headache following caffeine withdrawal was quite different from the migraine syndrome in the five migraine subjects studied, although the caffeine-withdrawal headache was accompanied by nausea in four of the migraine subjects and vomiting in one. In two subjects whose migraine is typically unilateral the caffeine-withdrawal headache was generalized. In two subjects the headache occurred on the opposite side of the head from their usual migraine. One subject has left-sided frontal migraine, but three caffeine-withdrawal headaches were bilateral and occipital in distribution. Three of these five subjects regularly have scotomas with their migraine headaches, but no such visual phenomena preceded or accompanied this experimental headache. Two migraine patients, however, had migraine headaches at the height

of the stimulant action of caffeine, and in one of these subjects this again occurred when another caffeine-withdrawal headache was attempted. It would thus appear that the true migraine headache is apt to occur during the period

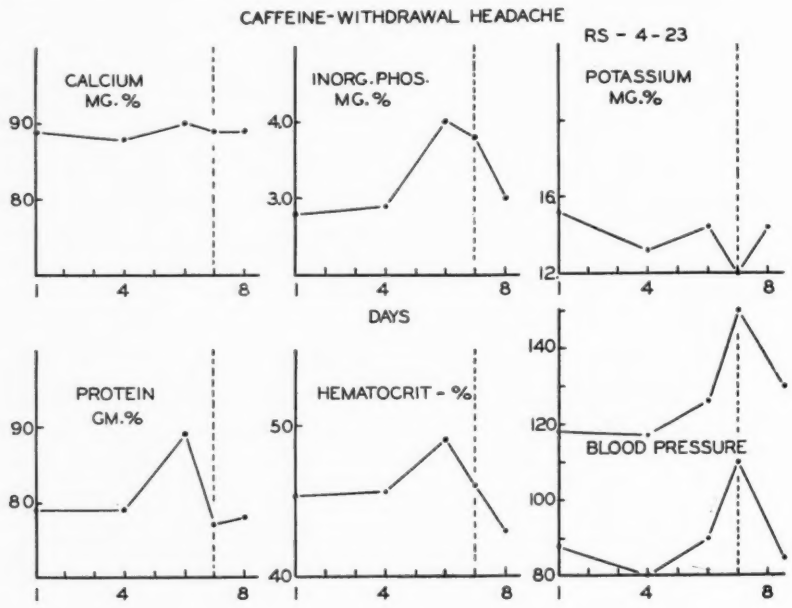

Fig. 1.

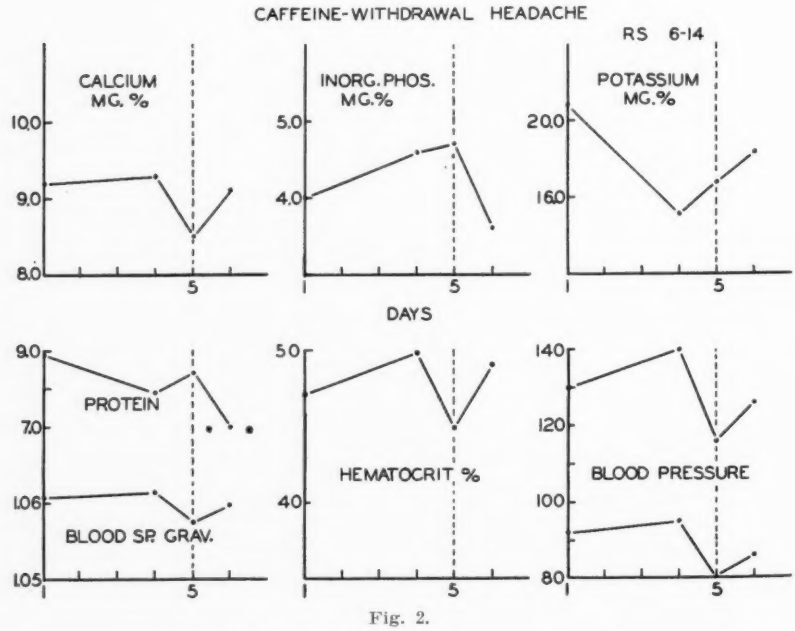

Fig. 2.

of caffeine overstimulation,* while the withdrawal of caffeine results in a distinct type of generalized headache. In a recent study[7] of migraine headache, we found no consistent changes in the serum calcium or phosphorus. A slight

*This led to an erroneous statement in the original Proceedings report.[1]

rise in hematocrit and total proteins was present at the time of the headache. The change in blood chemistry and blood volume found in this study would, hence, be another point of dissimilarity between the two types of headache.

That the caffeine-withdrawal headache is due to changes in cerebrospinal fluid pressure or volume is doubtful, since bilateral jugular compression which raises cerebrospinal fluid pressure does not intensify the headache. Amyl nitrite, however, relieves the headache during the short period of relaxation of the peripheral and splanchnic vascular tone. While the present data, due to the inadequacy of present methods, must necessarily be inconclusive, there are numerous findings that would be in accord with the hypothesis that this headache might result from an increase in effective arterial blood volume without a corresponding diminution in peripheral vascular tone, namely, the gradual onset of cerebral fullness, hyperemia of the ears, lowered serum proteins, the occurrence upon withdrawal of a diuretic drug, and the response to drugs and physical measures affecting headache.

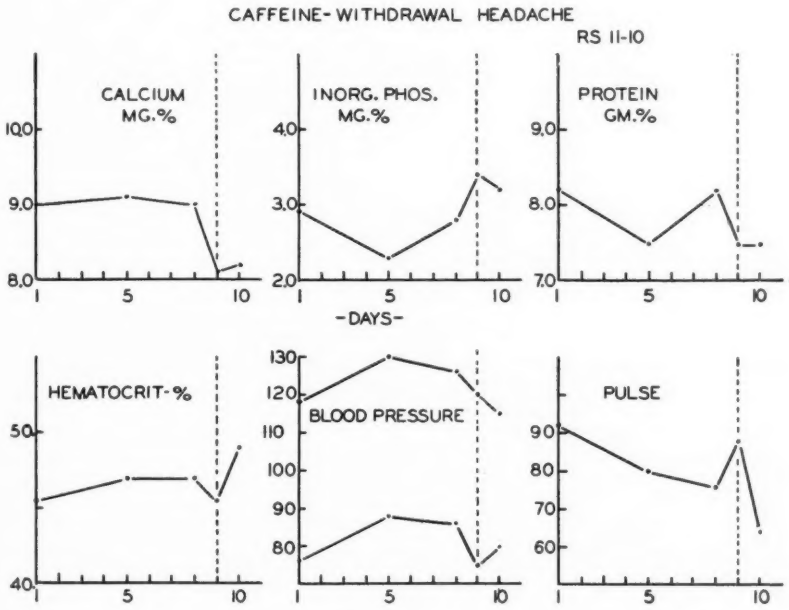

Fig. 3.

It is known from the work of Wolff[14, 15] that many headaches, including histamine and migraine headaches, are accompanied by an increased excursion of the cerebral blood vessels. This may occur reflexly or passively by changes in the volume or pressure of any one of the three semifluid media within the cranial vault, namely, the cerebrospinal fluid, the brain substance, or the blood. Since the first two media are relatively constant, and since the functional pathology of most headaches is known to reside in the vascular wall, the cause of the pain must be either a reflex dilatation of the blood vessels or an increased excursion of the vessels due to the two other factors which could affect the caliber of the cranial blood vessels, namely, the arterial blood volume or the pressure of the surrounding media. Bazett[16] has estimated the *arterial* blood volume from

anatomic measurements of the mesenteric vessels, and his observations indicate that the arteries probably contain a relatively small proportion (perhaps less than 20 per cent) of the total blood in a given region of the circulation. Thus, while the changes in blood volume demonstrated in this study are small, they may have exaggerated significance when combined with changes in cardiac output and peripheral vascular tone.

Since all factors must be considered, there remains the possibility that this headache might be due to direct or reflex dilatation of the cerebral vessels by the withdrawal of caffeine. Hirschfelder[17] has shown that caffeine tends to dilate the cerebral vessels if given intravenously. Oral caffeine is, however, believed to constrict the cerebral vessels, and this has been correlated by Sutherland and Wolff[18] with the observation that caffeine decreases the excursion of the cerebral blood vessels when used in the treatment of migraine headache.

The caffeine-withdrawal headache probably typifies one of the so-called "common headaches." In this respect it may be of value as an experimental headache. It may also provide a means of studying analgesic drugs by affording a method of producing predictable headaches. This study also provides a plausible explanation for the hitherto empirical addition of caffeine to many headache remedies.

The authors wish to express gratitude to the numerous subjects who aided in these experiments.

SUMMARY

In 55 per cent of thirty-eight trials on twenty-two subjects, headache as extreme in severity as the subjects had ever experienced was produced by the sudden withdrawal of caffeine. In 29 per cent of the trials the headache was definite, but did not require treatment. In 16 per cent of the experiments little or no headache resulted. The headache is without scotomas, slow in onset, central in origin, becoming generalized after four to six hours; and it may be accompanied by nausea and vomiting. In migraine subjects the headache differs from their typical migraine headache. The blood studies indicate that a lowered serum calcium, an elevated serum phosphorus, and possibly an increase in blood volume accompany the headache.

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THE ETIOLOGY OF THE MIGRAINE SYNDROME— A PHYSIOLOGIC APPROACH*

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THE possibility that blood volume changes might participate in the etiology of migraine headaches has not hitherto been investigated, although Lennox and Leonhardt[1] in 1937 suggested that alterations of "blood concentration" might play a dominant role in migraine headache. Established factors in the migraine syndrome which might be correlated with changes in blood volume are:

1. Onset at puberty and frequent relief at the menopause (correlating with onset and waning of cyclic activity of the sex hormones with their salt- and water-retaining properties).

2. Greater incidence in the female than in the male due to greater salt- and water-retaining power of estrogens compared to androgens.[2]

3. Menstrual migraine (perhaps due to sudden decrease in the estrogens with their salt- and water-retaining activity).

4. Relief of migraine by pregnancy due to the normal, concomitant increase in the blood volume with a high, maintained estrogen level.

5. Partial relief of the headache by the coal tar analgesics[3] and thyroid[4] therapy, both of which are known to produce a relative hydremia.

6. Marked relief by long-acting vasoconstrictor drugs, such as ergotamine tartrate and benzedrine sulfate (amphetamine).

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7. The occurrence of the headache with relaxation or a relative "let down" in activity.

These factors might be ascribed to an accompanying relaxation of peripheral-vascular tone without an increase in blood volume. Any altered physiology due to hereditary anomalies of the cerebral arteries would be precipitated at this time because of the decreased blood supply to the cranial area. It was the purpose of this study to determine whether headache of the migraine type is accompanied by relative changes in blood volume; at the same time other data, such as the blood pressure, pulse, serum calcium, phosphorus, and potassium, were obtained.

EXPERIMENTAL

1. *Relaxation Headache.*—The typical Sunday morning headache of the business man, the Monday headache of the clergy, the day-off headache of the nurse, and the post-examination headache of the student may all come under this classification. In order to determine if blood volume or electrolyte changes were involved in relaxation headaches, medical and graduate students were asked to come to the laboratory for blood sampling whenever they had a spontaneous headache. At that time a careful history was taken of the preceding sequence of sleep and activity to correlate any possible relaxation factor. In a series of twenty-six headaches studied, nine could be definitely correlated with relaxation (such as sleeping late). Blood samples were taken and analyzed for serum calcium, potassium, inorganic phosphorus, and proteins. The pulse, the reclining blood pressure (average of 3 to 4 trials), and hematocrits were also determined. A second series of observations and samples was taken after relief of the headache by caffeine and acetylsalicylic acid. If this therapy did not relieve the headache, the second blood sample was not obtained until one or two days had elapsed. Table I summarizes the findings in the nine headaches in which the subjects gave a history of relative relaxation. Ordinarily the serum proteins are the best index of changes in the blood volume, since they are usually constant. A rise in the serum proteins indicates a decrease in the blood volume at the time of the headache. Conversely, a lowered serum protein indicates a hemodilution. The hematocrit is much less reliable because it is changed by mobilization or storage of the red blood cells.

It is of interest to note that half of these subjects with relaxation headache have an appreciable rise in diastolic blood pressure at the time of the headache. With only two exceptions the change in the serum proteins indicates a relative hemoconcentration at the time of the headache. In the case of B. V. the first headache followed sleeping late after a hurried week-end motor trip. Accordingly, the same degree of sleepless activity was again artificially simulated, and the subject again slept late. Except for the blood pressure and the degree of the headache, the findings were almost identical. The first headache was, however, more severe, which happening might in some way be associated with the higher diastolic blood pressure.

2. *Migraine Headache.*—In a similar fashion, blood samples were obtained from migrainous patients suffering from typical migraine headache. Table II summarizes the findings in ten such headaches. These observations differ from

the findings in the relaxation type of headache in that five subjects showed a marked hypotensive level of blood pressure at the time of the headache with a return to normal on relief of the headache. The other six showed higher serum protein levels, thus indicating relative hemoconcentration at the time of the headache. In those cases that had no actual volume change, a relative inadequacy of the effective arterial blood volume is suggested by the decreased blood pressure, indicating a decreased peripheral-vascular tone. There was evidence of a lengthened blood-clotting time during the headache.

TABLE I

RELAXATION HEADACHE

	CA MG. PER 100 C.C.	P MG. PER 100 C.C.	K MG. PER 100 C.C.	PROT. GM. PER 100 C.C.	BLOOD PRESSURE	PULSE	HEMATOCRIT %	
1. B. V. 6/27	9.5	--	--	8.52	130/94	92	50.5	Headache
	9.5	--	--	8.31	134/84	88	50.6	Normal
2. B. V. 7/2	9.4	--	--	8.60	122/76	92	47.5	Headache
	9.2	--	--	8.30	116/78	96	49.2	Normal
3. L. I. 2/7	8.5	3.8	16.0	9.10	140/94	88	47.4	Headache
	--	--	17.5	8.17	138/98	82	48.0	Normal
4. S. S. 12/6	8.8	2.2	--	8.30	122/90	72	47.5	Headache
	9.0	2.3	--	8.60	110/88	68	48.3	Normal
5. R. S. 6/22	9.8	3.6	--	8.10	152/102	88	46.0	Headache
	8.7	2.6	--	7.70	122/85	68	44.2	Normal
6. I. I. 4/20	9.8	--	15.6	8.20	140/98	96	54.6	Headache
	9.8	--	17.9	8.00	126/88	102	45.5	Normal
7. R. D. 11/19	9.9	3.2	--	8.50	108/78	62	50.5	Headache
	9.6	3.7	--	7.75	114/78	60	47.0	Normal
8. P. S. 1/24	9.9	3.5	16.0	9.50	150/100	88	49.0	Headache
	9.6	3.6	16.0	8.80	126/80	54	43.5	Normal
9. R. D. 1/5	9.3	--	--	8.10	122/70	80	48.0	Headache
	9.4	3.5	--	8.10	112/68	56	47.1	Normal
Mean	9.6			8.55	132/89	84	49.0	Headache
	9.4			8.19	122/83	76	47.0	Normal

3. *Menstrual Migraine.*—A few patients with menstrual migraine have two types of headache. The first is a dull, generalized headache which may occur during the week preceding menstruation at the height of the estrogenic salt and water retention and may be due to a relative hemodilution. The second type, a typical migraine syndrome, occurs around the first day of menstruation and is perhaps due to the quick rebound phenomenon described by Harrop.[2] Table III summarizes the findings in five menstrual migraine headaches. It is evident again from these data that the only common factor at the time of the menstrual migraine headache is a decreased blood volume as indicated by a relatively higher serum protein level at the time of the headache. The subject

A. U. is of interest in that the menstrual migraine is accompanied by the same lowering of blood pressure which characterized a previous migraine attack that did not occur at menstruation. The average hematocrit is distorted by the final normal sample in No. 5 which was obtained two weeks after the headache. Menstrual blood loss might also affect the hematocrit readings at the time of the headache.

TABLE II

MIGRAINE HEADACHE

	CA MG. PER 100 C.C.	P MG. PER 100 C.C.	K MG. PER 100 C.C.	PROT. GM. PER 100 C.C.	BLOOD PRESSURE	PULSE	C.T.	HEMATOCRIT %
1. A. U. 4/8	10.0	--	16.8	8.20	95/70	84	4.50	42.5 Headache
	9.8	--	--	7.90	105/75	84	4.20	41.0 Normal
2. M. M. 6/13	9.8	--	--	9.20	138/98	82	4.20	48.5 Headache
	9.8	--	--	8.31	124/92	76	3.70	48.5 Normal
3. G. C. 4/24	10.8	--	14.4	8.50	134/90	104	3.80	48.5 Headache
	10.0	--	14.8	8.20	118/80	88	3.80	45.0 Normal
4. B. O. 5/19	9.3	--	19.1	8.81	80/58	68	4.50	45.0 Headache
	9.9	--	23.4	7.40	115/84	58	4.50	43.2 Normal
5. B. O. 6/26	9.4	--	25.3	8.12	96/66	70	4.75	43.0 Headache
	9.6	--	28.8	8.10	126/92	70	4.50	43.0 Normal
6. B. O. 8/2	9.4	3.0	--	8.28	90/62	84	6.00	44.0 Headache
	9.6	2.5	--	8.36	108/80	64	4.40	41.2 Normal
7. C. H. 10/25	8.4	4.0	--	7.94	90/70	76	--	45.5 Headache
	9.1	--	--	7.96	125/85	96	--	48.2 Normal
8. C. A. 8/20	9.8	--	--	9.32	120/85	80	--	47.0 Headache*
	9.4	--	--	7.80	115/92	100	4.70	44.0 Normal
9. L. U. 5/12	8.9	--	16.4	8.30	120/75	106	--	48.5 Headache
	8.6	--	16.4	8.00	118/88	76	--	46.7 Normal†
10. C. A. 4/22	10.0	2.5	13.6	8.44	135/95	114	--	47.2 Headache
	8.9	2.5	12.9	8.17	140/95	108	--	46.0 Normal
Mean	9.6			8.51	110/77	87	4.62	46.0 Headache
	9.5			8.02	119/86	82	4.18	44.7 Normal

*Sample taken after vomiting.
†One hour later.

DISCUSSION

While the extensive studies of Graham and Wolff and their colleagues[5] have indicated that cerebral pain is vascular in origin, they have not explained why the cerebral arteries should suddenly start dilating or why ergotamine, which acts mainly on the peripheral blood vessels, should decrease the excursion of the cerebral blood vessels. Our studies indicate that the excursion of the cerebral blood vessels may be traced to changes in arterial blood volume without compensatory changes in the peripheral vascular tone.

TABLE III

MENSTRUAL MIGRAINE HEADACHE

	CA MG. PER 100 C.C.	P MG. PER 100 C.C.	K MG. PER 100 C.C.	PROT. GM. PER 100 C.C.	BLOOD PRESSURE	PULSE	HEMATOCRIT %	
1. A. U. 2/27/39	9.3	--	19.1	8.00	94/74	84	42.0	Headache
	9.5	--	19.9	7.70	100/74	76	40.5	Normal
2. A. U. 3/22/39	9.9	3.0	--	8.00	98/68	92	42.0	Headache
	9.7	3.7	--	7.90	105/75	84	41.0	Normal
3. E. S. 11/22/38	8.5	2.8	--	7.85	122/82	68	43.2	Headache
	9.1	--	18.7	7.10	115/76	64	43.3	Normal
4. W. 2/8/39	9.0	--	17.5	8.70	118/80	76	42.2	Headache
	9.0	--	--	8.00	120/72	88	40.3	Normal
5. M. S. 12/16/39	9.8	3.8	--	8.94	120/85	80	42.0	Headache
	9.2	4.4	--	8.30	114/85	80	46.0?	Normal
Mean	9.3			8.30			42.3	Headache
	9.3			7.80			42.2	Normal

TABLE IV

COMPARISON OF BLOOD FINDINGS—CAFFEINE-WITHDRAWAL HEADACHE AND MIGRAINE HEADACHE

		BEFORE	DURING	AFTER
Serum Calcium	C. W. H. (17)	9.5 ± .4*	9.1 ± .4	9.4 ± .8
	Migraine (15)		9.5 ± .6	9.4 ± .4
Serum Phosphorus	C. W. H. (17)	3.2 ± .5*	3.6 ± .5	3.4 ± .4
	Migraine (15)		3.1 ± .5	3.2 ± .7
Serum Proteins	C. W. H. (17)	8.2 ± .4†	7.9 ± .4	8.1 ± .5
	Migraine (15)		8.4 ± .4	7.8 ± .3
Hematocrits	C. W. H. (17)	48.2 ± 2	46.7 ± 2	47.9 ± 2
	Migraine (15)		44.7 ± 2	43.8 ± 2

*Mg. per 100 c.c. ± Standard deviation.

†Gm. per cent ± Standard deviation.

The numbers in parentheses represent the number of subjects and patients used in compiling the respective means. The change in serum calcium and phosphorus found in subjects with caffeine-withdrawal headaches does not occur in migraine headaches. The blood volume trend, as judged by the changes in the hematocrits and serum proteins, is opposite in the two headaches.

In 1927 Loevenhart et al.[6] postulated that headache could arise from changes in volume or pressure of any one of the three fluid or semifluid media within the rigid confines of the skull. They defined these three semifluid media as the blood, the brain, and the cerebrospinal fluid. Headache resulting from changes in the volume of the brain and cerebrospinal fluid is well known and self-explanatory. In order to complete and clarify the discussion, we have summarized in Fig. 1 the known hydrodynamic factors which might possibly be involved in all types of headache. The pain-sensitive structures within the cranial vault, namely, the cranial blood vessels (arteries and veins), the dura, the tentorium cerebelli and the falx cerebri, are depicted. The various fluid pressure forces which might cause an increased excursion of the blood vessels or an abnormal stretch of the supporting membranes are also represented. Since the headache due to changes in cerebrospinal fluid volume and actual

brain volume is self-explanatory, the discussion will be limited to the changes in effective blood volume and peripheral vascular tone. While known data are not available on effective blood volume or cardiac output changes in nitrite or histamine headache, it is suspected that since these headaches do not occur

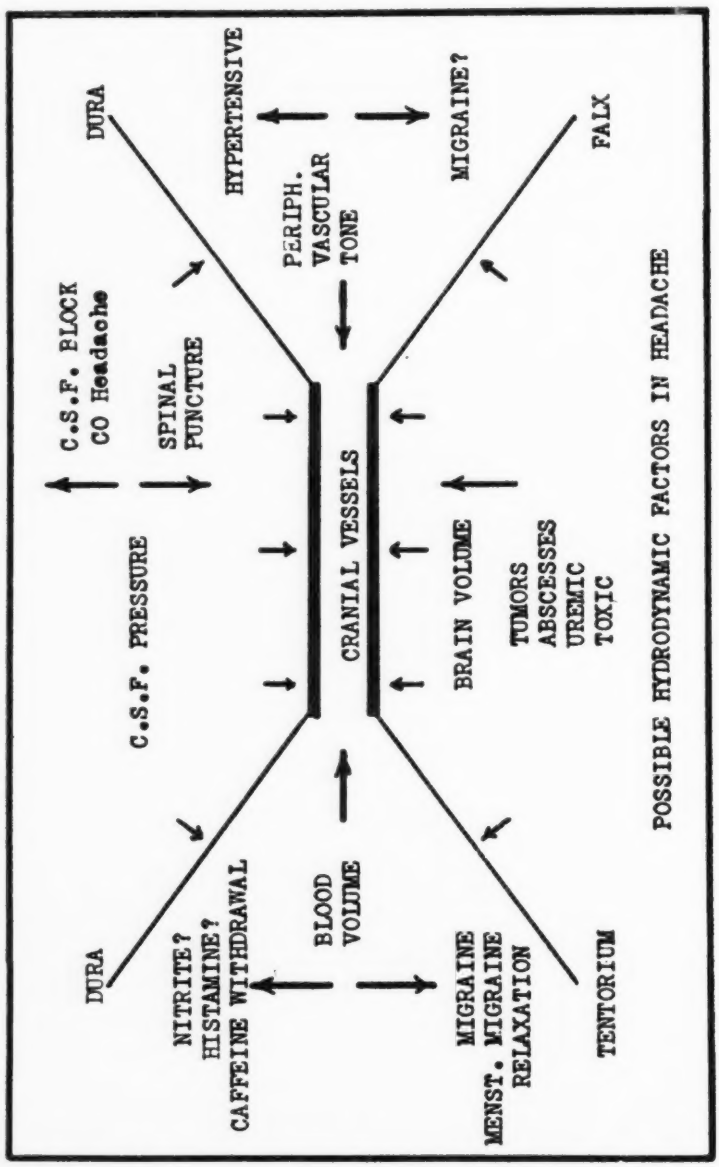

Fig. 1.

at the time of the hypotension from the drugs, they may be connected with the rise in blood pressure following the period of hypotension. If an increase in cardiac output occurs during the hypotensive period, the headache could be caused by a relative increase in the effective arterial blood volume at a time when the peripheral blood vessels are constricting. The data on "caffeine-withdrawal" headache[7] support the theory that this type of headache is accom-

panied by a relative increase in the effective blood volume. Conversely, these studies have substantiated the hypothesis that headache of the migraine and relaxation types is accompanied by a relative diminution in the blood volume.

Hypertensive patients are known to be frequently afflicted with so-called "tension headache." Experimental subjects in this laboratory have experienced similar headache with large doses of ephedrine or neosynephrin. It has been our experience that severe, persistent, frontal headache (occasionally with nausea and vomiting) occurs when normal subjects are given intramuscular doses of neosynephrin HCl in excess of 10 milligrams. This is relieved temporarily by inhalations of amyl nitrite; but complete relief does not occur until the blood pressure returns to normal. This could be accounted for by a sudden increase in peripheral vascular tone without an equal diminution in effective blood volume and cardiac output. A portion of the data in this study supports the theory that some migraine headaches may be accompanied by a marked decrease in peripheral vascular tone, and this is reflected in the semi-shocklike level of blood pressure obtained at the time of the headache.

The treatment of migraine headache should, then, be directed at increasing the blood volume or restoring an intense peripheral vascular tone by strong smooth muscle stimulants, such as ergotamine tartrate or amphetamine sulfate. When this is effectively accomplished, the excessive excursion of the cerebral blood vessels subsides with subjective relief.

SUMMARY

Data are presented which justify separation from the so-called "common headache" of two new types, namely, relaxation headache and "caffeine-withdrawal headache." The former is accompanied by a decreased blood volume, and the latter may be ascribed to a relative increase in the effective arterial blood volume. No consistent blood electrolyte changes occur with relaxation or migraine headache. The determination of the serum proteins and hematocrit during and after migraine headache indicates that the migraine syndrome is accompanied by a relative hemoconcentration. Many other known factors in this syndrome can be correlated with changes in blood volume and peripheral vascular tone.

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CHRONIC BRUCELLOSAL TYPE OF ANKYLOSING SPONDYLITIS

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DURING the past four years routine tests have been done on a number of patients with ankylosing spondylitis who came to the office for treatment. These tests, in addition to others, consisted of agglutination and opsonic index and skin test for brucellosis, bacterial complement fixation test with several strains of streptococcus antigens, especially hemolytic, viridans, nonhemolytic, and alpha prime *Streptococcus viridans;* and skin tests with a number of streptococcus strains, such as the hemolytic, viridans, alpha prime viridans, nonhemolytic, and anhemolytic streptococcus types.

In all there have been eighteen cases of ankylosing spondylitis. Of the eighteen patients two were women, indicating the usual ratio observed in ankylosing spondylitis of male to female, approximately 8 to 1.

Five patients gave quite positive skin, agglutination, and opsonic index tests for brucellosis. Rather interesting it was that those who gave strongly positive brucellosis tests in general gave essentially negative results on being tested for streptococcal antibodies and by skin test. Each patient had had his symptoms for quite some period of time. A diagnosis of ankylosing spondylitis was based upon a characteristic history, proper physical findings, and the demonstration by x-ray of changes in the vertebral ligaments indicative of ankylosing spondylitis, as well as of sacroiliac joint involvement.

Agglutination tests for brucellosis were carried out by the rapid plate method. The opsonic index test was done according to the method as elaborated by Huddleson and others; the skin test was carried out by injecting 0.1 c.c. of normal salt solution containing 10 million brucella organisms.

Streptococcal antibody tests were done in each case. It was felt that streptococcal skin tests should be performed in such a way that there would be no question, when a positive reaction was obtained, that definite hypersensitivity as a result of either past or present infection against the streptococcal strains was present. One-tenth c.c. of normal saline solution containing 10,000 organisms of the streptococci was injected intradermally, and observation of the skin test reaction was carried out over a period of at least forty-eight hours to as much as seventy-two or ninety-six hours.

A red cell sedimentation test was done in every case.

A review of the literature in respect to complications of either acute or chronic brucellosis, but especially of chronic or convalescent brucellosis, revealed some very pertinent findings in the foreign and American literature.

W. A. Bishop, Jr.,[1] makes some of the following important observations and quotes an extended review of the literature in his article. The result of his survey reveals:

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[1]J. Bone & Joint Surg. 21: 665, 1939.

1. "Spinal localization is the most common bone and joint complication of brucellosis."

2. "Localization may appear during any phase of the disease. Localization may be independent of a history of previous ailment. Localization as a complication is more common in the convalescent period."

3. He quotes Serio: "Local recurrence of the brucellosis is probably due to a lack of immunization to the organism."

4. "Localization occurs most often in the lumbar area."

5. "Frequent locomotor symptoms of chronic brucellosis are those of arthralgia, joint effusion, joint swelling, or a combination of arthralgia, joint effusion and joint swelling."

6. "The pathology has usually been described as an epiphysitis. Abscesses of vertebral bodies and/or vertebral discs have been described."

Bishop also quotes:

"Under pathology Feldman and Olson described in the hog irregular abscesses in the vertebral bodies and intervertebral discs. They were able to recover the organisms from ten of these cases. They described 0.5 to 3 cm. grayish-white, thick, pasty or caseous localized areas and occasionally sequestra. Certain lesions presented a crescent-shaped caplike inflammatory proliferation of osteogenic tissue bridging the intervertebral space on the ventral surface of the affected vertebrae. They concluded that the amount of ossification is variable depending on the duration of the lesion."

Bishop—"Their gross description of these lesions in hogs is compatible with the x-ray picture in human cases."

Bishop also quoted Serio as stating that chronic bony spurs on the anterior surface of the vertebral bodies would be noted as a part of the pathology and that occasionally calcification changes in focal areas would appear in the vertebral bodies instead of decalcification as is seen in tuberculosis. In other words, a characteristic zone of sclerosis instead of demineralization may occur at the original site of a brucellosis focus.

Bishop—"Serio states that these sclerotic changes accompany the infiltrative process, which infiltrative process gradually extends and may involve the entire vertebral body, and at times various processes of the vertebrae." Bishop also quotes: "There is variability of site and extent of lesions of the brucellosal complications in the spine. They may be confined to the disc and yet no narrowing of the disc or only very mild narrowing of the disc may occur. Often the vertebral body is normal or it may often be sclerotic or irregular. The original lesion may be masked by concomitant periosteal reaction, such as lipping or spurs on the anterior margin of the vertebrae." Bishop also makes specific mention from the literature of the fact *that occasionally an anteroposterior view of the spine will show the sclerotic processes following the contour of the intervertebral disc.* He also states that in cases in which the condition is more advanced constant and characteristic x-ray changes may be present as follows: "That all showed a reactive change, increased calcification of the bodies, circumscribed or diffuse, with proliferation of bone spurs across the interspace. Occasionally in the more severe cases deformity of vertebral body or *involvement*

of the articular facets will occur. Bony fusion of the bridges between the adjacent bodies is a common finding."

Bishop further quotes: "Hardy and Simpson, independently of each other, reported that one-third of patients with brucellosis had severe arthralgia. Straehn, in a thesis on an epidemic of undulant fever reported occurring in South Africa in 1911, recorded 268 cases in which joint swelling and effusion occurred in 15.3 per cent."

The symptoms of ankylosing spondylitis as a complication to chronic brucellosis are those of a case of infectious spondylitis in which pronounced rigidity of the spine, either locally or throughout its entire extent, plus either local or diffuse muscle spasm, will be present. Rigidity of the spine is especially present if the intervertebral articulations are involved.

The diagnosis of ankylosing spondylitis due to brucellosis must be based upon the history of back pain in patients who have had brucellosis in the past, in those who have some of the symptoms of chronic brucellosis, or in those who give strongly coordinated laboratory and skin test findings indicating the presence of either past or present chronic brucellosis. The agglutination and opsonic index tests are very important, especially the agglutination test. Often, as in other illnesses, the agglutination test may be negative or only very slightly positive, even though the disease process is present in the individual. The skin test should always be strongly positive, though cases are reported where a long-standing case of chronic brucellosis complication may have a negative skin test. The white blood cell count is usually normal or low. The patients may or may not have a temperature. The sedimentation rate may be accelerated. There should be negative findings for typhoid, if symptoms are acute, and negative findings for tuberculosis.

Of course the recovery of the organism from the lesions is the most important confirmatory finding that could be had. But its attempted recovery from chronic cases affords essentially a marked lack of positive results. It is a difficult procedure at the very best to recover organisms from a chronic brucellosis case. The usual findings that are present in a case of ankylosing spondylitis should be present, usually in a male, in the 15-to-45-year age group, with evidence of involvement of the sacroiliac and apophyseal joints, and the presence of calcification of some parts of the spinal ligaments or sacroiliac joints.

The development of the disease syndrome can be divided into three phases, the first phase being that of inexplainable symptoms and known as the prespondylitic phase; the second phase being one in which sacroiliac joint involvement can be demonstrated; and the third phase that in which definite and marked spinal rigidity is noted. The x-ray should be positive during the second and third phases. It will be negative during the first phase.

PATHOLOGY

Gunnar Edstrom[2] quotes the pathology of ankylosing spondylitis as being a chronic synovitis and tendinitis with round cell infiltration, hyperemia, and proliferation of the connecting tissue. These pathologic findings could be noted in any instance of an infection, whether such infection be by one or another known or unknown organism.

[2]Acta med. Scandinav. **104**: 396, 1940.

T. Lloyd Tyson[3] describes the pathology as "a primary synovitis of the intervertebral joints with subsequent calcification of the ligamentous structures and eventual rigidity of the spine." The synovitis of the small intervertebral joints may be accompanied by osteoporosis of the vertebral bodies. There is present also at all times synovitis of the sacroiliac joints. Synovitis of the costovertebral joints may be present. Later calcification of the long ligaments, of the ligamentum flavum, and the ligaments at the lateral border of the intervertebral discs will occur. He states that the pelvic and shoulder girdles may be affected. He notes especially that the small joints, when they are involved, are of a typical rheumatoid arthritis involvement. This observation was also reported, in the instance of two cases, by Goldfain.[4]

Jacques Forestier,[5] under the heading "The Importance of Sacro-Iliac Changes in the Early Diagnosis of Ankylosing Spondylitis," states: "Atrophic arthritis of the joints between the facets of the vertebrae in the inflammatory stage is followed by bony ankylosis." Later ossification of adjoining ligaments (ligamenta flava) and anterior and posterior vertebral ligaments occurs. He stresses the importance of noting by x-ray the presence of early changes in the sacroiliac joints. His description of the x-ray changes in the sacroiliac joints would seem to be the description of changes that are often seen in joints having rheumatoid arthritis type of pathology; namely, first there appears marginal decalcification, later there appear pyknotic formations, and finally in the last stage total synostosis.

TABLE I

CHRONIC BRUCELLOSIS SPONDYLITIS, ANKYLOSING TYPE

PATIENT	DIAGNOSIS	BRUCELLA AGG'N	BRUCELLA OPSONIC INDEX	BRUCELLA SKIN TEST	STREP. B.C.F. TEST	STREP. SKIN TEST	SED. TEST
No. 1, C. A. W.	Ankylosing spondylitis (Marie-Strümpell)	Complete 1-200	40% moderate	+4	–	–	–
No. 2, R. E. R.	Ankylosing spondylitis (Marie-Strümpell)	Complete	12% moderate		+4 Hemo. S. +4 APV S.	1+	+4
		1-200	52% mild	+4	+4 NH S.		
No. 3, G. G. (female)	Ankylosing spondylitis (Marie-Strümpell)	Complete 1-200	76% marked	+4	+4 Hemo. S. +4 +4 ?	1+	+2
No. 4, C. B.	Ankylosing spondylitis (Marie-Strümpell)	Marked 1-25 Partial 1-200	4% marked 20% moderate 68% mild	+4	–	–	+1
No. 5, A. R. S.	Ankylosing spondylitis (Marie-Strümpell)	Marked 1-50	4% moderate		+3 Alpha prime viridans		
		Partial 1-200	40% mild	+4		–	+3

COMMENT ON CASES QUOTED—TABLE I

CASE 1.—C. A. W. This patient lived in a small Oklahoma town where pasteurization was poorly practiced. There was a lack of other findings except a very small infected tonsil

[3]M. Clin. North America 21: 1755, 1937.
[4]J. LAB. & CLIN. MED. 27: 168, 1941.
[5]Radiology 33: 389, 1939.

remnant tissue, the removal of which did not help his symptoms. Improvement took place on general medical measures and bacterine therapy.

CASE 2.—R. E. R. The patient's home was in a small town in the panhandle of Texas, where no pasteurization of milk was practiced. In this case +4 tests for three strains of streptococci were present; however, the presence of strongly positive agglutination, opsonic index, and skin tests for brucellosis, plus long duration of trouble and suggestive positive history, justified labeling this case as due to brucellosis.

This patient did not respond well to bacterine therapy.

CASE 3.—G. G. The history of long continued illness, lasting several years, with episodes of fever, residence in a small town in the past, presence of same condition in a brother whom I examined but did not treat, plus strongly positive opsonic index, agglutination, and skin test for brucellosis justifies listing the patient as brucellosal spondylitis, ankylosing type. She responded quite well to bacterine therapy.

CASE 4.—C. B. This patient was living on a farm when his rheumatism began with arthralgias. Removal of his tonsils did not help. Loss of weight and weakness were prominently present. The negative antibody and skin tests for streptococci and strongly positive opsonic index and skin tests and moderately positive agglutination tests justify a diagnosis of chronic brucellosal spondylitis, ankylosing type. The patient's response to treatment has been only moderately favorable thus far.

CASE 5.—A. R. S. This patient lived in the panhandle of Texas. He lived on a farm. Milk was not pasteurized or boiled. His sister also had brucellosis. In a period of eighteen months on routine arthritic management and brucella bacterine his sedimentation test had become normal, and patient is free of symptoms.

CONCLUSIONS

1. Of eighteen cases of ankylosing spondylitis, five can be listed as being caused by chronic brucellosis in this series.

2. Ankylosing spondylitis is a symptom complex. The cause may be an infection which can be either determined, as apparently the five cases quoted could be, or nondeterminable in our present stage of knowledge.

3. Chronic brucellosis, like syphilis or tuberculosis, is protean in its manifestations. It may reveal itself in different ways. Apparently one of the complications of chronic brucellosis may be that of a rheumatoid type of arthritis, which when limited to the spine presents the symptom complex of ankylosing spondylitis.

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THE SIGNIFICANCE OF THE GROSS CHARACTER OF THE SPUTUM IN THE PROGNOSIS OF PNEUMOCOCCIC PNEUMONIA*

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RECENT studies concerning the important role of edema in the severity and spread of the pneumonic process in the lungs[5, 6, 9, 10, 11, 14] have stimulated our interest in the character of the gross sputum in pneumonia. At necropsy we noted a widespread, heavily infected zone of inflammatory edema in the lungs of patients who had produced large quantities of sputum during life. On the other hand, a narrow, well-defined edema zone at the periphery of the advancing consolidation was present in patients who raised small amounts of more tenacious sputum before death. On the basis of these observations the former cases were classified as "wet" lungs and the latter were designated as "dry" lungs. The characterization of cases as dry or wet lungs was then extended to include all pneumonia patients in whom the gross sputum was examined at daily intervals during the course of the disease.** In the present study of 651 cases, exclusive of Type III,† these findings have been correlated with the number of pneumococci per oil immersion field in Wright-stained smears of sputum,[1, 3] the type of pneumococcus, blood culture, leucocyte count, age of the patient, duration of the disease, the extent of involvement, and the outcome of the pneumonia.

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**The following report has been restricted to patients who produced rusty sputum. Satisfactory specimens were obtained from 90 per cent of the cases due to Types I through VIII; the remaining 10 per cent, who were either uncooperative or unable to produce rusty sputum, were excluded from the study.

†The outcome in Type III pneumonia is determined primarily by the amount of capsular polysaccharides formed in the sputum and lungs.[2, 4, 13]

METHOD

On admission to the hospital the patients were requested to cough and expectorate into a container. Observations concerning the ease of production and the quantity of sputum were made at the bedside. The specimens were then taken to the laboratory, examined under a strong light, and portions were removed for sputum counts in the manner previously described.[1, 3] The classification of patients as wet or dry lungs was usually, but not always, easy. Those patients who produced homogeneously rusty, watery to thin gelatinous sputum in considerable quantities and with relative ease on coughing were classified as wet lungs. Those who, upon vigorous coughing, raised small amounts of viscid sputum, which was either homogeneously rusty or mixed with mucoid or mucopurulent material, were called dry lungs. In dehydrated patients the final classification was deferred until adequate fluids had been administered. In borderline cases the consistency of the sputum was the chief factor in determining classification.

RESULTS

Studies of 678 cases of pneumococcic pneumonia reported in detail elsewhere[3] have shown that the fatality rate was only 2 per cent when the average number of pneumococci per oil immersion field in Wright-stained smears of sputum did not exceed 10 (Group A). The fatality was 9 per cent when the sputum count ranged between 11 and 30 per field (Group B), 30 per cent when 31 to 75 organisms were present (Group C), and 77 per cent when the number exceeded 75 per field (Group D). The relation of the gross character of the sputum to the microscopic counts and to the outcome of the disease is presented in Table I. The fatality rate was 6 per cent for all cases classified as dry lungs and 23 per cent for those designated as wet lungs. When the comparison was restricted to patients whose sputum counts fell within a similar range, the fatality rates for the dry and wet lungs were essentially the same and were primarily influenced by the number of pneumococci present; however, only 7 per cent of the dry and 41 per cent of the wet lungs had more than 30 pneumococci per field in their sputa. The close relationship between the sputum count and the character of the pneumonic exudate is further emphasized by a consideration of the percentage of total cases within a given sputum group which were classed as dry or wet lungs: thus 85 per cent of the cases in Group A were dry and 15 per cent were designated as wet lungs. Corresponding figures for Group B were 45 and 55 per cent; for Group C, 28 and 72 per cent; for Group D 2 and 98 per cent, respectively. The significance of these data will be discussed.

In a previous study[3] it was also found that type, qualitative bacteremia, leucocyte count, and duration of the disease before therapy were relatively unimportant factors in prognosis when compared with the number of pneumococci in the sputum. Since 80 per cent of the patients with more than 30 organisms per field were characterized as wet lungs, it seemed important to determine the relation between the gross sputum and other prognostic factors. In Table II the cases have been classified according to the presence or absence of bacteremia. Of the total group of 651 patients studied, 59 per cent were dry and 41 per cent were wet lungs. Among those with negative blood cultures, however, the in-

cidence of dry and wet lungs was 75 per cent and 25 per cent, respectively. These figures were reversed in the group with positive blood cultures, where dry lungs were found in only 26 per cent and wet lungs in 74 per cent. It would appear, then, that blood stream invasion may be dependent upon the formation of large amounts of exudate in the edema zone of the lungs.

TABLE I

RELATION OF DRY AND WET LUNGS TO SPUTUM COUNTS

SPUTUM GROUP	DRY LUNG				WET LUNG				TOTAL CASES		
	CASES		DEATHS		CASES		DEATHS				
	NO.	% OF TOTAL	NO.	% FAT.	NO.	% OF TOTAL	NO.	% FAT.	NO.	% DRY	% WET
A 10 or less	262	69	3	1	47	18	2	4	309	85	15
B 11-30	92	24	10	11	111	41	8	7	203	45	55
C 31-75	27	7	7	30	68	25	21	31	95	28	72
D Over 75	1	0	1	100	43	16	32	74	44	2	98
Totals	382	100	21	6	269	100	63	23	651	59	41

TABLE II

RELATION OF DRY AND WET LUNGS TO BACTEREMIA

SPUTUM GROUP	NEGATIVE BLOOD CULTURE		POSITIVE BLOOD CULTURE		TOTAL CASES		
	CASES		CASES			BLOOD CULTURE	
	NO.	% OF TOTAL	NO.	% OF TOTAL	NO.	% NEGATIVE	% POSITIVE
Dry lung	327	75	55	26	382	86	14
Wet lung	112	25	157	74	269	42	58
Totals	439	100	212	100	651	67	33

TABLE III

RELATION OF DRY AND WET LUNGS TO TYPE*

TYPE	DRY CASES		WET CASES	
	NO.	% OF TOTAL	NO.	% OF TOTAL
I	80	54	69	46
II	60	55	48	45
VII	67	60	45	40
VIII	45	57	34	43
Others	130	64	73	36
Totals	382	59	269	41

*Type III not included.

The relationship between the gross character of the sputum and the type of pneumococcus responsible for the disease is shown in Table III. A breakdown of the cases revealed that the percentage of dry and wet lungs in Types I, II, VII, and VIII pneumonia did not deviate significantly from the mean of 59 and 41 per cent, respectively. Thus there was no evidence from this study that the formation of large amounts of exudate in the lung is a property peculiar to certain types of pneumococci.

The relationship between leucocyte counts and the gross character of the sputum is presented in Table IV. Among the total group of 483 cases with leucocytosis the incidence of dry and wet lungs was 64 and 36 per cent, re-

spectively, as compared with 43 and 57 per cent in the 254 patients with leucocyte counts below 10,000 per cubic millimeter. These figures reveal that there is a definite trend toward leucopenia in wet lung cases, but the differences were too small to suggest that a deficit of leucocytes is the chief factor in the formation of the edema zone in the lung.

The relationship between the gross character of the sputum and the duration of the disease on admission to the hospital is shown in Table V. Only 33 per cent of the total cases were seen more than 96 hours after the pneumonia had developed. The incidence of dry and wet lungs, however, remained approximately the same irrespective of the duration of the disease. It was concluded from these data that the length of time the patient was ill played little, if any, role in the formation of large quantities of exudate in the lung.

TABLE IV

RELATION OF DRY AND WET LUNGS TO LEUCOCYTE COUNT

SPUTUM GROUP	10,000 AND OVER		5,000 TO 9,999		LESS THAN 5,000		TOTAL CASES		
	CASES		CASES		CASES		NO.	LEUCOCYTE COUNT	
	NO.	% OF TOTAL	NO.	% OF TOTAL	NO.	% OF TOTAL		% OVER 10,000	% BELOW 10,000
Dry lung	311	64	49	42	16	44	376	83	17
Wet lung	172	36	69	58	20	56	261	66	34
Totals	483	100	118	100	36	6	637	76	18

TABLE V

RELATION OF DRY AND WET LUNGS TO DURATION OF DISEASE BEFORE THERAPY

SPUTUM GROUP	LESS THAN 48 HOURS		48 TO 95 HOURS		96 TO 143 HOURS		144 HOURS AND OVER		TOTAL CASES		
	CASES		CASES		CASES		CASES			HOURS	
	NO.	% OF TOTAL	NO.	% OF TOTAL	NO.	% OF TOTAL	NO.	% OF TOTAL	NO.	% UNDER 96	% OVER 96
Dry lung	124	59	130	59	75	60	47	55	376	68	32
Wet lung	85	41	89	41	50	40	39	45	263	66	34
Totals	209	100	219	100	125	100	86	100	639	67	33

In a previous study[3] it was noted that the degree of involvement and the age of the patient were factors which significantly influenced the fatality rate independent of the sputum count. In the present study, a close correlation was observed between the character of the gross sputum and the extent of consolidation. The incidence of wet lungs was only 34 per cent in the cases with consolidation confined to one lobe as compared to 64 per cent in those with multiple lobe involvement. While the reversal of the percentages was significant (Table VI), it was felt that the multiple lobe involvement, like bacteremia, was the result of, rather than the cause of, the outpouring of exudate into the bronchi and trachea. In this respect the character and quantity of the exudate might have contributed to the higher fatality rate associated with multiple lobe involvement.[3]

An analysis of the gross character of the sputum with reference to age is presented in Table VII. The incidence of wet lungs increased from 32 per cent in patients below 30 years to 44 per cent in those between 30 and 54 and finally to 51 per cent in the older age group. The trend revealed by these figures suggests that the character and quantity of the pneumonic exudate depend to some extent upon the age of the patient.

TABLE VI

RELATION OF DRY AND WET LUNGS TO INVOLVEMENT

SPUTUM GROUP	ONE OR LESS LOBES		MORE THAN ONE LOBE		TOTAL CASES		
	CASES		CASES			LOBES	
	NO.	% OF TOTAL	NO.	% OF TOTAL	NO.	% ONE OR LESS	% MORE THAN ONE
Dry lung	325	66	57	36	382	85	15
Wet lung	169	34	100	64	269	63	37
Totals	494	100	157	100	651	76	24

TABLE VII

RELATION OF DRY AND WET LUNGS TO AGE OF PATIENT

SPUTUM GROUP	AGE 5 TO 29 YEARS		AGE 30 TO 54 YEARS		AGE 55 YEARS AND OVER		TOTAL CASES		
	CASES		CASES		CASES			AGE	
	NO.	% OF TOTAL	NO.	% OF TOTAL	NO.	% OF TOTAL	NO.	% UNDER 30	% OVER 30
Dry lung	124	68	218	56	40	49	382	32	68
Wet lung	58	32	169	44	42	51	269	21	79
Totals	182	100	387	100	82	100	651	28	72

DISCUSSION

The classification of cases according to the gross character of the pneumonic sputum is a simple procedure which appears to be of considerable aid in prognosis. An overwhelming majority of the patients with dry lungs showed less than 30 pneumococci per field (93 per cent), a negative blood culture (86 per cent), a favorable leucocyte response (83 per cent), and consolidation which was confined to a single lobe (85 per cent). The relatively mild nature of the pneumonia which could be predicted from the appearance of the gross sputum was further substantiated by the fact that 94 per cent of the patients recovered irrespective of therapy. On the other hand, 41 per cent of the cases which were designated as wet lungs showed more than 30 pneumococci per field in the sputum; 58 per cent had bacteremia; 34 per cent had leucocyte counts below 10,000; and 37 per cent had involvement of more than one lobe on admission to the hospital. The pneumonia in these patients was more severe as shown by the higher fatality rate (23 per cent). As a general rule the clinical response to sulfonamides was slower in the cases classed as wet lungs.

The observations of Loescheke[9] first drew attention to the significance of the edema zone in the transfer of pneumococci from one portion of the lung to another. His findings were soon corroborated by other workers.[6, 10, 11, 14] The experiments of Robertson and Hamburger[11] were of special interest to us because

of their conclusion that the severity of experimental pneumonia in the dog was directly related to the wetness of the lung. They believed that "metastatic lesions occur only when the primary lesion is sufficiently edematous to permit escape of fluid exudate through its main bronchus into that of another lobe or lobes, the direction of flow being determined largely by gravity." The bronchi of animals severely ill with extensive pneumonia yielded considerable quantities of a fluid exudate containing from 5 to 50 billion organisms per cubic centimeter. On the other hand, the exudates from animals with well-localized lesions were usually viscid and contained relatively few pneumococci. From these observations, which were substantiated by our own necropsy findings, it seems likely that the amount and gross character of the sputum in human pneumonia reflects the state of the lung, copious wet sputum indicating an extensive area of inflammatory edema, a dry sputum indicating a more localized lesion. Such a hypothesis furnishes us with a plausible explanation for the high incidence of bacteremia and multiple lobe involvement in patients who have wet lungs, since the presence of a large edema zone would permit adequate opportunity for blood stream invasion or for metastatic spread to other lobes. It seems to us that the wet lung is a more likely cause of bacteremia than the result of the blood stream invasion, as has been suggested by Kempf and Nungester.[8]

The question as to why one patient should produce large quantities of rusty sputum and another relatively little remains unanswered. While an increase in the proportion of wet lungs occurred with advancing age and with leucopenia, it was felt that these were accessory rather than primary factors determining the character of the pneumonic exudate. The close correlation between the number of pneumococci and the nature of the gross sputum may be interpreted in one of two ways. It is possible that the outpouring of fluid into the alveoli represents an allergic response to the presence of a few pneumococci,[7] thus creating an environment which facilitates the rapid multiplication of the organisms; however, it seems more likely that the amount of exudate is directly proportional to the quantity of edema-producing substance elaborated by the large numbers of pneumococci present in the lung. This concept is supported by (a) the work of Sutliff and Friedemann,[12] who demonstrated such an edema-producing substance in filtrates of young pneumococcus cultures, and by (b) the studies of Hamburger and Robertson,[6] in which either localized or diffuse edematous lesions were experimentally induced at will by varying the dosage of pneumococci. The solution to this problem must await further study.

SUMMARY

The gross character of rusty sputum in 651 cases of pneumococci pneumonia was correlated with standard prognostic criteria and the outcome of the disease. As a result of observations made at necropsy, those patients who produced small amounts of a viscid rusty sputum were classified as "dry" lungs, whereas those who raised large quantities of watery to gelatinous, homogeneously rusty sputum were classified as "wet" lungs. The pneumonia in the former group was relatively mild, while the disease in the latter group was considerably more severe, as shown by fatality rates of 6 per cent and 23 per cent, respectively, as well as by significant differences in the number of pneumococci

in the sputum, the extent of consolidation, and the incidence of bacteremia and leucopenia. It was concluded that the gross character of the sputum was of distinct value in the prognosis of pneumococcic pneumonia. The significance of the observations with reference to the pathology of the pulmonary lesions is discussed.

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CLINICAL CHEMISTRY

THE COMPARATIVE ACCURACY OF THE CLOSED CIRCUIT BEDSIDE METHOD AND THE OPEN CIRCUIT CHAMBER PROCEDURE FOR THE DETERMINATION OF BASAL METABOLISM*

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SUFFICIENT data have been accumulated by the investigations of the Child Research Council at Denver to establish standards of basal metabolism on children between the ages of 2 and 16 years. The first report of this work by Lewis, Kinsman and Iliff[1] gives the results obtained prior to April, 1936, and presents tentative standards for both boys and girls from 2 to 12 years old inclusive. Since the appearance of this publication, the question has been raised as to whether these standards may be used when determinations are made with a bedside type of apparatus. No report of a comparison of the results obtained with a chamber procedure and with a bedside apparatus has been found in the literature, although several studies[2-4] have been made on adults both with the open circuit and the closed circuit methods but where a mask or a mouthpiece and a nose clip have been used in both cases. In the present study determinations of the basal metabolism of both children and adults made with an open circuit chamber procedure have been compared with those obtained with a closed circuit bedside type of apparatus.

The open circuit chamber method was that used to establish standards of basal metabolism on children of the Child Research Council. The procedure is fully described in the publication of Lewis, Kinsman, and Iliff.[1] It should be noted that with this method a 15 per cent protein metabolism has been assumed and that the calorific value per standard liter of oxygen, which varies with the respiratory quotient found, has been obtained by reference to the chart of Michaelis.[5]

The closed circuit apparatus employed was a No. M 84 McKesson Metabolor, to which the subject was connected by means of a mask (face inhaler) or with a mouthpiece and nose clip. A McKesson face inhaler, a McKesson mouthpiece, and a Sanborn nose clip were the types of accessories used. The mouthpiece and nose clip were used invariably on adults and in about half of the tests on children. Those cases in which the mask (face inhaler) was used on children are indicated by an asterisk (*) in Table I. The quantity of oxygen consumed in

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A preliminary report of this study was made at the meetings of the Colorado-Wyoming Academy of Science at Golden, Colorado, in November, 1941 (Iliff, Alberta, Anna Marie Duval and Robert C. Lewis, J. Colo.-Wyo. Acad. Sci. 3: 56, 1942).

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a given length of time was measured, and a value of 4.825 calories per standard liter of oxygen was used to calculate the heat production. In order to determine the consumption of oxygen, check tests of four or more minutes in length were procured in all cases. If the oxygen consumption showed a deviation of more than 4 per cent from the mean when two determinations were made, at least one more test was obtained. For three or four tests to be acceptable, it was required that the oxygen consumption in each case fall within 5 or 7 per cent, respectively, of the mean of all tests.

Alcohol check determinations were made on both types of apparatus during this study. With the chamber method these determinations are made routinely about four times a year. The procedure and the results obtained are given in other publications.[1, 6] The amounts of oxygen consumed and of carbon dioxide produced by the burning of a known quantity of alcohol in the chamber have been found to fall within ±2 per cent of the theoretical values. Twenty-nine determinations with the closed circuit apparatus were made during this study by means of the alcohol check apparatus of Jones,[7] and in these the amount of oxygen consumed varied from the theoretical values by −3.1 to +3.0 per cent with an average deviation of +0.4 per cent.

EXPERIMENTAL PROCEDURE

The experimental procedure followed was to make a determination of basal metabolism in the chamber either immediately preceding or immediately following one with the closed circuit bedside apparatus. Such comparisons were made in 65 instances on 36 children (19 boys and 17 girls) between the ages of 2 years, 8 months, and 15 years, 3 months, and in 55 instances on 25 adults (8 men and 17 women). All subjects were in a postabsorptive condition when the tests were performed. The children had had a normal amount of sleep and were brought from home in an automobile by one of the workers in the metabolism laboratory. The adults had had approximately eight hours' sleep on each of the two nights preceding a test and came to the laboratory by automobile.

In the majority of experiments, a determination was made first with the chamber and then with the McKesson Metabolor. After the subject was comfortable and relaxed, the lid of the chamber was closed and, when the preliminary period (thirty minutes for children and forty-five minutes for adults) had elapsed, samples of air were collected for analysis. The lid of the chamber was then raised, and while the subject remained quiet the body temperature, the pulse rate, and the respiratory rate were taken. The determination with the McKesson Metabolor was then made.

When the bedside method was used first, it was necessary for the subject to remain quiet fifteen or twenty minutes longer than when the reverse order was followed. Since many of the children were inclined to become restless when the experimental period was thus prolonged, only four experiments in which the determinations with the closed circuit apparatus preceded that with the chamber were conducted on children. With adults, 23 experiments were made in this sequence. After the subject rested in the chamber for the preliminary period (thirty minutes for children and forty-five minutes for adults), the consumption of oxygen was measured with the McKesson Metabolor. When satisfactory determinations

TABLE I

COMPARATIVE RESULTS OBTAINED FOR THE BASAL METABOLISM OF CHILDREN WITH THE OPEN CIRCUIT CHAMBER PROCEDURE AND THE CLOSED CIRCUIT BEDSIDE APPARATUS (McKESSON)

SUBJECT, SEX	AGE		WEIGHT (KG.)	HEIGHT (CM.)	BODY SURFACE (SQ. M.)	CAL./HR./SQ. M.		DIFFERENCE (PER CENT)
	YR.	MO.				BY CHAMBER METHOD	BY MC KESSON METHOD	
7(♂)	2	8	13.7	92.8	0.580	47.6	50.7	+6.5
82(♂)‡	4	2	15.2	104.6	0.660	49.6	51.6	+4.0
53(♀)	4	7	17.7	110.6	0.735	48.2	48.2	±0.0
87(♀)	4	8	14.5	102.5	0.645	45.8	44.9	-2.0
	4	11	14.6	104.7	0.650	46.9	48.5	+3.4
67(♀)	4	11	18.8	106.5	0.735	53.9	51.3	-4.8
71(♀)	5	0	15.3	109.8	0.690	49.6	49.4	-0.4
	5	3	16.1	112.3	0.720	49.0	48.5	-1.0
97(♀)	5	4	19.3	113.4	0.780	46.8	49.5	+5.8
37(♀)	5	7	22.2	119.7	0.860	49.3	51.7*	+4.9
	6	2	23.0	124.2	0.895	46.9	46.9	±0.0
46(♀)	5	9	20.5	118.5	0.825	45.1	47.7	+5.8
29(♂)	5	9	21.0	114.7	0.815	55.8	53.5	-4.1
	6	0	21.2	115.8	0.825	53.4	51.9	-2.8
	6	3	21.3	117.6	0.830	53.2	53.5	+0.6
21(♀)	6	1	18.8	113.8	0.775	45.8	46.2*	+0.9
	6	5	18.7	114.2	0.775	45.8	47.7*	+4.1
27(♂)‡	6	7	22.6	121.8	0.875	50.8	49.8*	-2.0
3(♀)	6	7	16.0	112.7	0.715	48.3	50.2	+3.9
	6	11	16.9	114.3	0.740	48.9	49.5	+1.2
23(♂)	6	8	22.9	119.7	0.870	48.1	44.5	-7.5
95(♀)‡	7	7	25.9	124.6	0.940	46.5	44.3	-4.7
	7	10	27.1	126.6	0.970	46.6	46.9	+0.6
93(♂)	7	7	25.1	127.0	0.940	48.4	47.2*	-2.5
	8	1	27.0	130.3	0.985	45.9	44.1*	-3.9
91(♂)	8	0	22.8	127.7	0.910	49.1	48.9*	-0.4
	8	6	23.6	130.6	0.935	45.4	46.1	+1.5
92(♂)	8	5	29.7	137.1	1.070	43.4	44.6	+2.8
G(♂)‡	8	6	25.2	131.8	0.970	47.2	45.9*	-2.8
	8	9	25.6	133.4	0.985	47.0	48.0*	+2.1
J(♂)	9	0	23.4	129.6	0.925	47.7	47.4	-0.6
	9	4	24.7	130.7	0.960	45.2	47.4	+4.9
	9	10	25.4	133.5	0.980	45.5	46.3	+1.8
81(♂)	9	1	26.0	131.5	0.980	46.5	44.7*	-3.9
	9	8	27.6	134.9	1.020	49.4	46.6*	-5.7
83(♂)‡	9	8	30.6	144.4	1.130	41.7	39.7*	-4.8
80(♂)	9	9	22.5	131.2	0.920	43.3	44.3	+2.3
73(♀)	9	11	25.6	130.1	0.970	43.1	44.2*	+2.6

*In these cases a mask was used; in all other cases a mouthpiece and a nose clip were employed.

†In these experiments the determination with the McKesson Metabolor preceded the observation with the chamber procedure.

‡These children required a preliminary period of training before satisfactory determinations were obtained.

TABLE I—CONT'D

SUBJECT, SEX	AGE		WEIGHT (KG.)	HEIGHT (CM.)	BODY SURFACE (SQ. M.)	CAL./HR./SQ. M.		DIFFERENCE (PER CENT)
	YR.	MO.				BY CHAMBER METHOD	BY MC KESSON METHOD	
70(♀)	10	2	34.6	150.0	1.225	38.5	36.7*	-4.7†
	10	3	34.6	149.1	1.225	38.1	37.1*	-2.6†
B(♂)‡	11	2	45.8	149.3	1.405	40.3	42.3*	+5.0
E(♀)	11	5	40.1	147.1	1.260	44.2	41.9*	-5.2
	11	11	46.7	143.3	1.370	39.5	39.5	±0.0
C(♂)	11	8	45.7	154.2	1.375	45.9	48.4*	+5.4
	11	11	46.3	150.7	1.390	48.3	46.6*	-3.5
48(♂)	12	9	40.5	157.9	1.355	42.6	41.7*	-2.1
	13	1	42.2	159.7	1.390	38.4	40.3*	+4.9
12(♂)	12	11	35.6	145.5	1.210	37.1	38.2*	+3.0
	14	4	40.0	153.1	1.315	39.2	41.6*	+6.1
	14	6	40.8	155.1	1.340	43.0	40.0*	-7.0
	14	9	42.4	157.5	1.380	42.7	40.5*	-5.2
34(♀)	13	0	67.7	164.2	1.740	37.5	37.9*	+1.1
20(♂)‡	13	3	42.8	162.0	1.410	42.5	41.4*	-2.6
	13	6	44.7	164.4	1.450	40.9	41.7*	+2.0
33(♀)	13	8	59.4	158.5	1.605	35.8	35.4*	-1.1
	14	0	62.0	160.3	1.645	34.0	35.7*	+5.0
15(♂)‡	14	0	43.1	162.8	1.425	43.0	41.4*	-3.7
	14	3	47.1	165.1	1.495	43.1	42.7	-0.9
	14	5	47.3	166.8	1.510	40.2	40.0	-0.5†
	14	5	47.5	166.7	1.510	38.7	41.3	+6.7
	14	5	47.3	166.8	1.510	39.2	39.4	+0.5†
	14	6	47.5	166.9	1.510	38.9	40.7	+4.6
11(♀)	14	4	45.6	170.7	1.510	37.9	37.8*	-0.3
6(♀)	15	0	45.8	168.6	1.500	33.6	32.9*	-2.1
	15	3	46.3	168.8	1.510	33.4	34.3*	+2.7

had been obtained, the bedside apparatus was disconnected and the lid of the chamber was closed. After allowing a period of fifteen or twenty minutes to permit the carbon dioxide in the chamber to reach equilibrium, samples of air were collected for analysis.

EXPERIMENTAL RESULTS

The experimental results show an excellent agreement between the values obtained by the bedside method and by the chamber procedure. The calories per hour per square meter of body surface found by each of the two methods are given in Table I for children and in Table II for adults. These tables also give the difference between the values obtained by the two methods in each case in terms of percentage deviation from the determination made in the chamber. These deviations are shown graphically in the accompanying histograms (Fig. 1 for children, and Fig. 2 for adults). The results with the closed circuit bedside method show a deviation from those with the open circuit chamber procedure of from -7.5 to +6.7 per cent (mean variation = +0.2 per cent) for children and of from -8.6 to +9.3 per cent (mean variation = +0.1 per cent) for adults. The order in which the determinations were made had no effect on the results, for

TABLE II

COMPARATIVE RESULTS OBTAINED FOR THE BASAL METABOLISM OF ADULTS WITH THE OPEN CIRCUIT CHAMBER PROCEDURE AND THE CLOSED CIRCUIT BEDSIDE APPARATUS (MCKESSON)

SUBJECT, SEX	AGE (YEARS)	WEIGHT (KG.)	HEIGHT (CM.)	BODY SURFACE (SQ. M.)	CAL./HR./SQ. M.		DIFFERENCE (PER CENT)
					BY CHAMBER METHOD	BY MC KESSON METHOD	
HB(♀)	21	59.6	169.5	1.685	30.4	31.0	+2.0
		59.6		1.685	30.0	29.6	-1.3
		59.8		1.685	30.4	29.9	-1.6
BM(♀)	21	53.1	157.4	1.520	31.0	33.5	+8.1
		53.1		1.520	34.4	33.2	-3.5†
		53.7		1.530	35.3	33.0	-6.5
NM(♂)	22	65.0	172.6	1.775	33.7	33.1	-1.8
		65.7		1.780	34.3	32.6	-5.0†
		65.5		1.780	33.3	34.9	+4.8
GM(♂)	22	66.2	183.6	1.870	34.7	36.7	+5.8
		67.5		1.880	35.9	37.5	+4.4†
		67.7		1.885	35.9	36.5	+1.7
FM(♀)	22	50.2	167.2	1.555	30.6	30.6	±0.0†
		50.1		1.550	32.4	32.3	-0.3
LP(♀)	23	61.8	175.6	1.755	30.0	28.8	-4.0
		61.5		1.750	28.6	28.7	+0.3†
AJ(♀)	23	57.8	160.5	1.600	31.7	31.5	-0.6†
		56.9		1.590	30.3	28.9	-4.6
RC(♂)	25	73.0	190.3	1.995	32.7	32.1	-1.8†
		73.4		2.000	31.9	32.1	+0.6
GD(♂)	26	76.6	179.2	1.955	36.1	36.7	+1.7
		76.4		1.950	34.6	33.8	-3.5†
AW(♂)	26	67.2	183.5	1.880	35.3	33.3	-5.7
RW(♂)	26	72.3	178.8	1.900	32.9	31.0	-5.8
DH(♀)	26	57.9	170.9	1.670	30.0	30.5	+1.7
		57.6		1.670	29.5	30.3	+2.7†
AK(♀)	27	60.3	169.4	1.690	29.3	30.8	+5.1
		60.4		1.695	29.6	30.0	+1.4†
AMD(♀)	27	53.8	158.6	1.540	30.1	32.9	+9.3
		53.1		1.530	31.1	33.4	+7.4†
		54.0		1.540	30.5	31.5	+3.3
LW(♀)	27	50.5	163.4	1.530	31.1	29.6	-4.8†
		50.5		1.530	30.9	29.5	-4.5
JR(♀)	27	55.3	159.7	1.565	28.2	30.8	+9.2
		55.4		1.565	29.5	28.2	-4.4†
		56.4		1.580	29.6	30.5	+3.0
EL(♀)	27	51.6	166.4	1.565	29.0	31.4	+8.3†
		51.6		1.565	29.4	28.0	-4.8
		51.7		1.565	30.4	30.3	-0.3†
HD(♀)	29	50.9	166.6	1.560	32.2	33.3	+3.4
		51.1		1.560	30.5	32.2	+5.6†
AI(♀)	31	61.9	175.1	1.755	33.0	31.0	-6.1
		62.7		1.765	31.4	31.6	+0.6†
VT(♀)	31	62.4	171.0	1.730	30.4	31.3	+3.0
		62.8		1.735	30.9	30.4	-1.6†

†In these experiments the determination with the McKesson Metabolor preceded the observation with the chamber procedure.

TABLE II—CONT'D

SUBJECT, SEX	AGE (YEARS)	WEIGHT (KG.)	HEIGHT (CM.)	BODY SURFACE (SQ. M.)	CAL./HR./SQ. M.		DIFFERENCE (PER CENT)
					BY CHAMBER METHOD	BY MC KESSON METHOD	
MMF(♀)	32	73.6	170.1	1.850	34.8	34.4	−1.1
		73.1		1.845	33.2	33.2	±0.0†
CW(♂)	35	68.3	172.8	1.810	36.3	36.2	−0.3†
		68.3		1.810	35.6	34.4	−3.4
TM(♂)	37	66.0	167.8	1.750	35.5	32.5	−8.4†
		65.2		1.740	34.4	34.6	+0.6
GMK(♀)	38	66.2	168.4	1.755	33.8	30.9	−8.6†
		66.6		1.760	30.5	31.0	+1.6
KW(♀)	43	74.4	157.9	1.760	32.6	34.0	+4.3†
		74.5		1.760	35.0	35.5	+1.4

it was found that, whichever procedure was followed, the value obtained with the bedside apparatus was in some instances higher and in some lower than that procured with the chamber method. The tests in which the bedside procedure was performed first are indicated by a dagger (†) in Tables I and II.

The coefficient of correlation of the calories per hour per square meter of body surface as determined by the two methods has been calculated by the formula, $r = \dfrac{\dfrac{\Sigma xy}{n} - m_x m_y}{\sigma_x \sigma_y}$, where x represents the calories per hour per square meter as determined by the chamber method; y, the calories per hour per square meter as determined with the McKesson Metabolor; n, the number of tests; m, the mean; and σ, the standard deviation of the calories per hour per square meter. The formula, $\sigma_r = \dfrac{1 - r^2}{\sqrt{n}}$, has been used to determine the standard error of the coefficient of correlation. The value found for the coefficient of correlation of the calories per hour per square meter of body surface as determined by the two methods is 0.94 with a standard error of ±0.013 for children and 0.81 with a standard error of ±0.046 for adults. These values show an excellent correlation between the two methods both for children and for adults.

Table I shows also that reliable determinations of basal metabolism can be procured with a bedside apparatus on children under 8 years of age. This is evident, since the results of the determinations with the portable apparatus on the sixteen younger children are as satisfactory as are those on the children over 8 years old. A preliminary period of training to procure successful determinations was found necessary with three of the children between 2 and 8 years, but five of the twenty older children also required a period of training. These eight children are indicated by a double dagger (‡) in Table I. Several other investigators, who have used a mask or a mouthpiece and a nose clip, have reported satisfactory results on children under eight years old. Götteche[8] procured reliable determinations on four children and Farr[9] on seven children between 3 and 8 years of age. In the large series of de Bruin[10] there were 196

children between the ages of 1 and 8 years on whom successful observations were made. Both Farr and de Bruin reported that a preliminary period of training was frequently necessary. The results of the present study and of the other investigations cited would seem to refute the contention of Talbot, Wilson, and Worcester[11] that children of 7 years or younger cannot be tested with a portable apparatus.

The fact that satisfactory determinations of basal metabolism on children have been obtained both with the chamber procedure and with the bedside apparatus shows that factors other than the type of apparatus used determine the success to be attained when children are studied. Among these factors are those inherent in the subject on whom the determinations are being made. All workers in the field of energy metabolism realize how essential it is to obtain the cooperation of the subject to the end that he will remain quiet throughout the determinations. They will also agree that it is more difficult to secure cooperation from children, and particularly from young children, than from adults. This difficulty may be overcome, however, if the persons who conduct the test have experience with children and are patient. It is first necessary to win the child's confidence and then to enlist his help in making the determination a success. When full cooperation is procured in this way, a satisfactory determination of basal metabolism can be obtained.

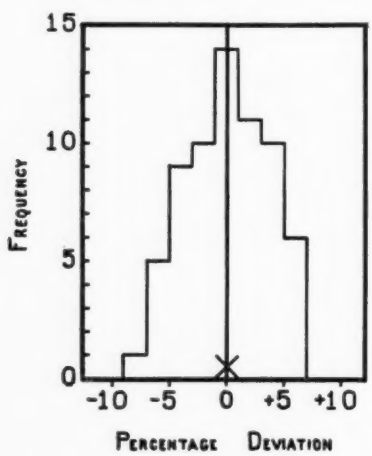

Fig. 1.—Frequency distribution of the percentage deviation of the calories per hour per square meter obtained on children with the closed circuit bedside method from those found with the open circuit chamber procedure. X represents the mean percentage deviation with regard to sign.

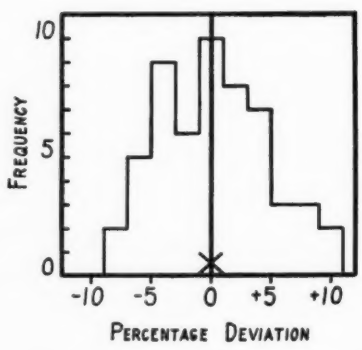

Fig. 2.—Frequency distribution of the percentage deviation of the calories per hour per square meter obtained on adults with the closed circuit bedside method from those found with the open circuit chamber procedure. X represents the mean percentage deviation with regard to sign.

Reference has been made in the beginning of this report to the standards of basal metabolism established by the investigations of the Child Research Council at Denver. These standards which were first published by Lewis, Kinsman, and Iliff[1] in 1937 have been slightly modified by the inclusion of data obtained since that time, and may be found with tentative standards for the fourteenth, fifteenth, and sixteenth years in a forthcoming article by Lewis, Duval, and Iliff.[12] From the results of the present study it may be concluded that these

standards for basal metabolism of children are equally reliable whether a closed circuit bedside method or a chamber procedure is used.

SUMMARY

1. Either immediately preceding or immediately following a determination of basal metabolism with an open circuit chamber method, 65 determinations on 36 children between the ages of 2 and 16 years and 55 determinations on 25 adults were made with a closed circuit bedside apparatus.

2. An excellent correlation between the results obtained by the two methods was found both for children and for adults.

3. Evidence is presented that, contrary to general belief, reliable determinations of basal metabolism on children under 8 years of age may be procured with a bedside type of apparatus.

4. The importance of obtaining cooperation from children in order to insure the successful determination of basal metabolism and the means employed to accomplish this end are discussed.

5. The standards for the basal metabolism of children established by the Child Research Council of Denver with an open circuit chamber procedure are shown to be suitable for use when the determination is made with a portable closed circuit apparatus.

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LABORATORY METHODS

GENERAL

BIOPSY OF BONE MARROW PERFORMED BY A NEW AND SIMPLE INSTRUMENT*

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THE examination of marrow usually obtained from the sternum by aspiration has become an almost universal practice in the diagnosis of hemopoietic disorders, disturbances of the reticulo-endothelial system, certain infectious diseases, and primary or metastatic neoplasms within the marrow cavity. The advantages of the aspiration method lie in the ease in which the test may be performed, its relative freedom from discomfort or inconvenience to the patient, and the technical similarities between the stained films of aspirated marrow and those prepared from circulating blood. On the other hand, the aspirated material may not be representative of the quantitative relationship between cellular elements within the marrow; in myelosclerosis, no material may be obtainable; and in hypoplastic states, the information gained by aspiration is of uncertain significance. Moreover, films prepared from the mixture of marrow and blood secured by aspiration permit no conclusions regarding the anatomical relationship of the marrow elements.

The limitations of the aspiration method of sternal biopsy have led many hematologists to place chief reliance upon the examination of material obtained by trephine. A number of instruments have been derived for this purpose and that employed by Dameshek[1] is particularly satisfactory. Nevertheless, the minor surgical procedure involved, with incision and resulting scar, has precluded the general application of the method for diagnostic purposes.

It is the object of this communication to describe an instrument designed to obtain a small specimen of marrow with no more effort and actually less discomfort to the patient than are involved in the ordinary aspiration procedure. This device, with minor modifications, is also adapted to the securing of biopsy material from tumors and soft tissues. This instrument consists of two needles with stylets, an outer guiding needle of 14 gauge (*A*), and an inner trephine needle of 17 gauge (*B*), Fig. 1.

*From the Thomas Henry Simpson Memorial Institute for Medical Research, University of Michigan.
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The external guiding needle should be 2 to 3 cm. in length. One end of this needle has a sharp beveled point, while the other end has a hollow head with a slot into which the projection of the head of the stylet fits. This arrangement keeps the point of the needle and the point of the stylet at the same cutting angle. The purpose of the guiding needle is to cut (split) through the skin and subcutaneous tissue without excising them, and to direct the inner trephine needle to the desired position.

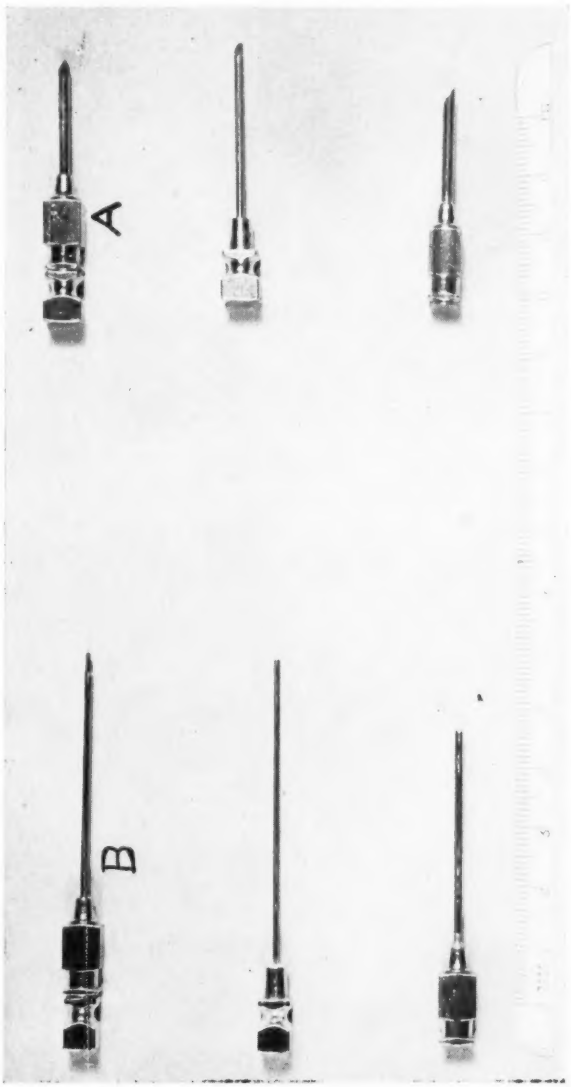

Fig. 1.—Trephine needle for bone marrow biopsy.

The internal or cutting needle, whose diameter is equal to that of the stylet of the external needle, is designed to be slipped within the external needle after the stylet is removed. The length of the two needles may be varied, but the relation must be such that the shaft of the internal needle is 5 mm. longer than the entire length of the external needle. The tip of the inner needle possesses four

saw teeth. The inside surface of the tip is cone-shaped or converging, so that the tissue becomes wedged into the needle (like a cork stopper into a flask) after being cut, and remains within the needle when the latter is removed.

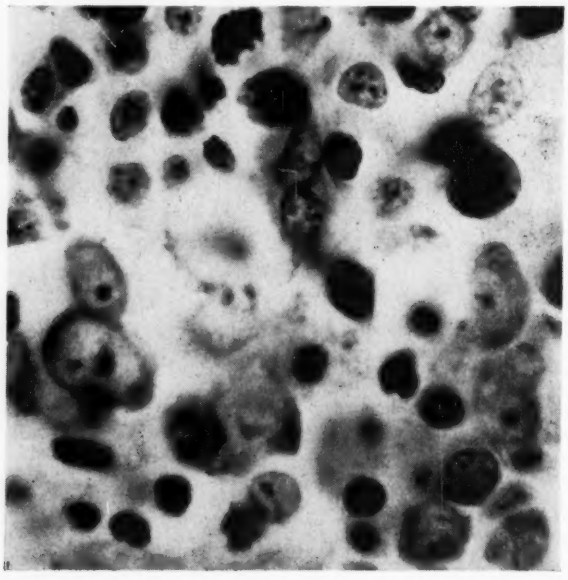

Fig. 2.—Marrow section, physician 57 years of age, suffering with subleucemic myeloblastic leucemia. No diagnostic cells were found in the blood film or aspirated material. Subsequently the disease entered a leucemic phase.

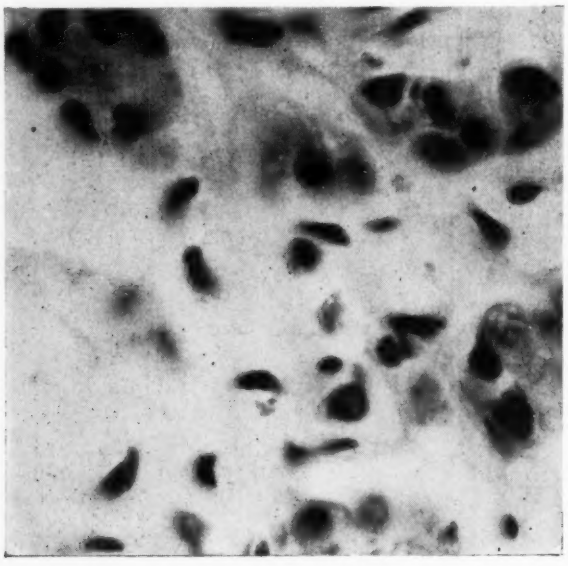

Fig. 3.—Marrow section, female 57 years of age, exhibiting nests of carcinoma cells not demonstrable by the aspiration technique. The patient was suffering with carcinoma of the breast.

The head of the internal needle is hollow, but without a slot. A standard adapter or syringe would fit into the heads of both needles. The stylet has no

projection on its head, but its stem is slightly longer than the inner needle, thus extending through the tip. This additional length serves to express the biopsy

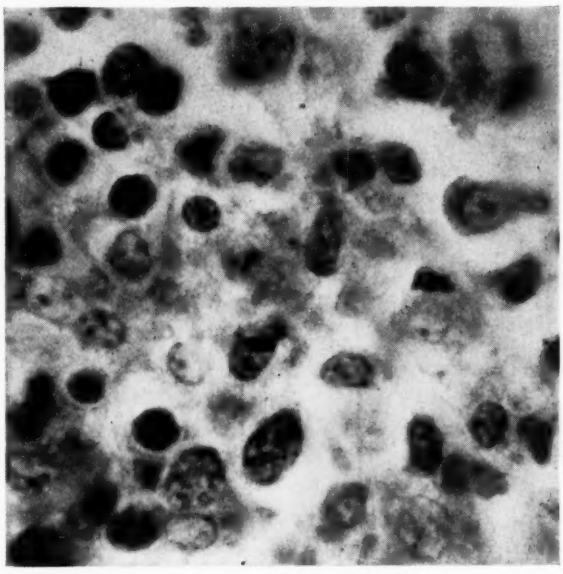

Fig. 4.—Marrow section, 44-year-old male, with multiple myeloma. Atypical plasma cells and plasmablasts were also seen in the films of aspirated marrow.

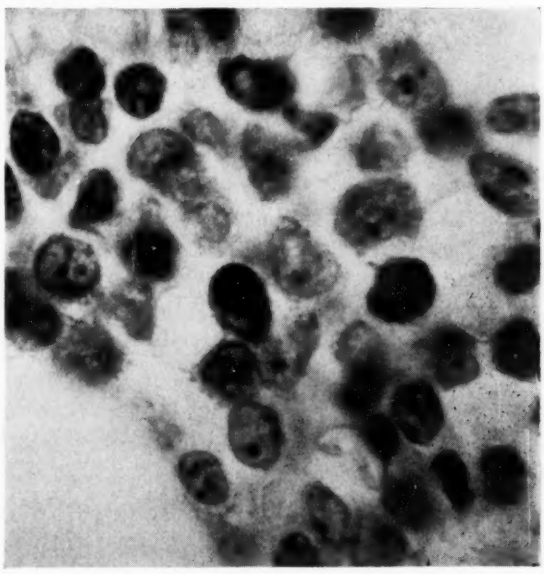

Fig. 5.—Marrow section, female 65 years of age, with subleucemic reticulosis (histiomonocytic leucemia). In this case the aspiration films were diagnostic.

material out of the inner needle and also protects the cutting tip from injury when the inner needle is inserted into the outer needle.

TECHNIQUE FOR STERNAL TISSUE BIOPSY

1. Cleanse the skin over the desired site, usually opposite the third rib, with a surgical antiseptic solution.
2. Infiltrate the skin, subcutaneous tissue, and periosteum with a local anesthetic (procaine 1.5 per cent).
3. Insert outer needle with stylet in place, with the tip of needle in direction of the face of patient at an angle of about 45 degrees, through the skin, subcutaneous tissue, and periosteum, until point just engages the anterior lamella of the sternum.
4. Remove stylet of the outer needle.
5. Insert cutting needle with stylet into outer needle.
6. Remove stylet of inner needle.
7. While holding the head of the outer needle with fingers of the left hand, turn the head of the cutting needle with fingers of the right hand backwards and forwards through a half circle, at the same time exerting gentle pressure. A sudden release of resistance signifies the entrance of the cutting tip into the sternal cavity. The cutting action with slight pressure should be continued until the needle has entered the sternal cavity for a distance of 2 to 3 mm. The needle should then be revolved several times without further insertion in order to insure detachment of the plug from the surrounding marrow.
9. Remove the inner needle.
10. Remove the outer needle.
11. Cover the point of insertion with a collodion dressing.
12. Insert stylet into the inner needle and push out the biopsy core into a bottle containing fixative solution.

It is usually also desirable to prepare films of aspirated marrow and for this purpose material obtained from the trephine hole is not, as a rule, satisfactory, because of the admixture of excessive amounts of blood. We have preferred to anesthetize a sufficiently large area to permit insertion of an aspiration needle about 1 cm. below the point of introduction of the trephine. The additional manipulation causes the patient no further discomfort, and in fact, he is usually not aware that two punctures have been made.

For fixation we have employed 100 c.c. of Zenker's solution with 5 c.c. of glacial acetic acid added just before using. After twenty-four hours, no further decalcification is required. The specimens are then washed, dehydrated, cleared, and imbedded in paraffin in the usual manner. The sections are cut with a thickness of five microns. The most satisfactory stains which we have used are Giemsa-Wolbach[2] and Kingsley.[3]

The procedure has been carried out, in conjunction with aspiration, on one hundred twelve patients representing a wide variety of hematologic conditions, and suspected primary or metastatic neoplasms of the marrow. A detailed analysis of the observations made on these patients, as well as a much larger series in which aspiration alone was done, will be published in a later communication.

Only rarely is positive diagnostic information obtained by the trephine method and not by aspiration, but such cases do occur. (Figs. 2 and 3.) The

accompanying photomicrographs were made with a 2 mm. apochromatic objective and 10× compensating ocular.

SUMMARY

1. The advantages and limitations of the aspiration method of marrow biopsy are discussed.

2. An instrument is described which enables a small specimen of marrow to be obtained by trephine without a skin incision. Photomicrographs of marrow sections secured by this method are presented.

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THE NONSPECIFICITY OF THE SERUM-OPACITY TEST FOR CLOSTRIDIUM WELCHII*

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INTRODUCTION

THERE has long been a need for the rapid identification of *Clostridium welchii* in specimens taken from traumatic cases with symptoms and signs of gas gangrene. In time of war this need is much greater and the available laboratory facilities are usually fewer. It is quite natural therefore in the present emergency to attempt to utilize the reaction described by Nagler,[1] since it is supposed to be specific for the Type A toxin of *Cl. welchii*. It is the purpose of this communication to call attention to the error of using such a test, which in reality is entirely nonspecific; because other antisera will prevent the reaction, and many common aerobes ordinarily present in contaminated wounds may also give a positive reaction.

HISTORICAL

In 1939 Nagler[1] pointed out that *Clostridium welchii* grown in a mixture of human serum and broth produced opacity in the medium. Usually, but not always, a curd was formed which on centrifuging rose to the surface of the fluid, while the bacterial cells deposited on the bottom of the tube left a clear fluid intervening. This reaction could be entirely prevented by growing the organisms in a similar mixture which contained in addition *Cl. welchii* antitoxin. All strains of *Cl. welchii* tested gave the reaction, but all other anaerobes used did not. These facts suggested the reaction was specific for *Cl. welchii* and that it was probably due to Type A toxin, since all four types (A, B, C, and D of Wilsdon's classification[2]) gave the reaction.

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Recently Hayward[3] reported the use of this reaction in the rapid identification of *Clostridium welchii* in clinical material submitted for examination. She also tested 174 strains of known non-welchii anaerobes and found that 9 gave positive results or 5.1 per cent. She also found considerable variation in the amount of inoculum required to obtain a positive reaction when using known strains of *Cl. welchii*, the required number of organisms varying from as low as 400 to as high as 200,000. Whereas Nagler originally found the reactions to be positive in 16 to 18 hours' cultivation, she found some strains required as much as three days to produce opacity or coagulation. There is no evidence that she tested any of the common aerobes which would likely be present in clinical material submitted for routine bacteriologic examination. To date we have found no literature indicating any one has studied this reaction employing aerobes in place of anaerobes except the report by Nagler,[1] who used a pseudoanthrax organism.

TABLE I

PRODUCTION OF SERUM OPACITY BY VARIOUS ORGANISMS AND THE INHIBITION OF THE REACTION BY USE OF NONSPECIFIC ANTITOXINS

ORGANISMS		ANTISERUM USED				
		NONE	*Cl. welchii*	*Cl. tetani*	*Vib. septique*	*C. diph.*
Cl. welchii	H	+	−	−	−	
	M	+	−	−	−	
	C	+	−	−	+	
	G	+	+	−	+	
	3628	+	−	−	−	
	3630	+	+	−	+	
	3631	+	−	−	+	
	3626	+	+	+	+	
	3627	+	+	+	+	
	DR 2	+	−	−	+	
	SR_2 2	+	−	−	+	
	6504	+	−	−	−	
	6506	+	−	−	−	
	6509	+	+	+	+	
	7907	+	−	−	−	
Cl. novyi	7658	+	−	−	−	
Cl. putrificum	4996	+	−	−	−	
Cl. botulinum	687	+	−	−	−	
Cl. botulinum	6060	+	−	−	−	
Cl. bifermentans	638	+	−	−	+	
Cl. oedematis maligni	461	+	−	−	−	
Proteus vulgaris		+	−	−	−	−
Escherichia coli	5	+	−	−	−	−
Escherichia coli	105	+	+	−	−	−
Aerobacter aerogenes		+	−	−	−	−
Pseudomonas aeruginosa		+	−	−	−	−
Diphtheroid	R 1194	+	+	−	−	−
Staphylococcus aureus	S	+	−	−	−	−
	PLR 1	+	−	−	−	−
	PLR 2	+	−	+	+	−
	a	+	−	−	−	−
	b	+	+	−	−	−
	R 1216	+	+	−	−	−
	KH 2	+	−	−	−	−
	KH 3	+	−	−	−	−
Staphylococcus albus	K 3	+	−	−	−	−
	S	+	−	−	−	−
	C	+	−	−	−	−
Staphylococcus citreus		+	−	−	−	−

EXPERIMENTAL

The technique described by Nagler was followed in detail except where other antiserums were used in place of *Cl. welchii* antitoxin. The tubes were incubated eighteen hours in the anaerobic jar. The controls always showed good growth and the reactions were definite.

Table I shows the production of a positive reaction with many aerobes and anaerobes other than *Cl. welchii* and the inhibition of the reaction with various types of antitoxins. It also shows the inability of *Cl. welchii* antitoxin to prevent the reaction. The data very clearly demonstrate the nonspecificity of the reaction.

CONCLUSION

The serum opacity reaction for the identification of *Cl. welchii* may be prevented by heterologous antitoxins. The reaction is produced by many common aerobes and anaerobes and these reactions may likewise be prevented by the use of nonspecific antitoxins. The reaction should not be used for the identification of *Cl. welchii*.

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DOSING OF POWDERED DRUGS TO SMALL ANIMALS*

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I HAVE noticed in several recent publications that workers use the stomach tube for dosing small animals with powders such as sulfapyridine. This is neither accurate nor easy, and is risky when animals are weak or ill as a result of experimental procedures.

The instrument described in Fig. 1 costs nothing. With it, small animals such as guinea pigs, rabbits, and cats can be dosed safely and expeditiously.

The end of the barrel of a broken-tipped 1-c.c. tuberculin syringe is cut off and ground flat. The outer edge is beveled slightly so as to avoid a cutting edge. A light metal sleeve, which can be cut from the guard in which large veterinary hypodermic needles are packed, is fitted over the plunger and adjusted so that ½ mm. of the plunger projects when this is pushed home into

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the barrel. The sleeve simply acts as a stop. An instrument without the sleeve, and with the plunger cut to the proper length, is not nearly so handy. The making takes about fifteen minutes.

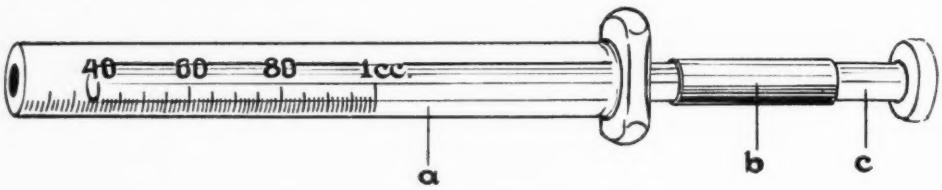

Fig. 1.—a, barrel; b, sleeve; c, plunger.

TABLE I

WEIGHTS OF TWENTY SUCCESSIVE FILLINGS

TO ADMINISTER 100 MG.	TO ADMINISTER 200 MG.
99.5	203.0
100.0	203.0
100.0	199.0
101.0	202.0
103.0	201.5
102.5	199.0
100.0	203.0
103.5	201.0
101.5	203.0
102.5	201.0

The instrument, which is merely a small balling gun, is filled by withdrawing the plunger to a mark calibrated for the required dose (one of the original gradations), and dabbing the end of the barrel into a small container of the powder until the powder reaches the end of the plunger. The end of the balling gun is pushed over the back of the animal's tongue, and the load discharged. A good degree of accuracy is obtained by this filling method. Table I gives the weights of twenty successive fillings for administering 100 and 200 mg. doses of sulfapyridine to guinea pigs.

AN IMPROVED METHOD FOR THE ISOLATION OF MANNITOL-FERMENTING SHIGELLAE*

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INDUCED by the unsatisfactory results encountered with the preserving fluids in prevalent use for Shigellae, search was instituted for more adaptable media. Among others, the fluid of Bangxang and Eliot[1] was tested for such suitability. Although announced over two years ago, this medium apparently had not obtained extensive application, and in order to ascertain its effectiveness, a test was made with laboratory strains of Shigellae, and with feces infected, in vitro, with Shigellae.

The subcultures from Bangxang and Eliot's medium inoculated in this manner proved rich in Shigellae for a period of at least four days. Because sulfa drugs may inhibit the growth of Shigellae, the fluid was tested also for its suitability in preserving Shigellae in the feces of persons treated with such drugs. The necessity to counteract sulfa drugs presented itself as an urgent task. The amount of 5 mg. p-aminobenzoic acid per 100 c.c. of the fluid was found sufficient to abolish the inhibitory effect on any microbes of sulfanilamide, sulfathiazole and sulfaguanidine when these drugs were given in the usual therapeutic concentrations. The amount of 5 mg. per 100 c.c. was chosen as a standard supplement of Bangxang and Eliot's preservative. P-aminobenzoic acid in this concentration does not stop the growth of mannitol-fermenting Shigellae.

It was found that the optimal efficacy of the preserving fluid is guaranteed at a pH around 8 and that dried bile may be substituted for sodium desoxycholate. Therefore the medium was prepared in the following modification:

Dissolve 10 Gm. of sodium citrate, 10 Gm. of peptone, 5 Gm. of sodium desoxycholate (or 6 Gm. of dried bile) and 9 Gm. of sodium chloride in 1 L. distilled water. Add 3 c.c. of a 1 per cent monosodium phosphate solution, neutralize with n/10 sodium hydroxide. Add 0.05 Gm. p-aminobenzoic acid; adjust pH to 8 with sodium hydroxide; tube, or put in containers filling up one-third of the glass. Sterilize in flowing steam.

For the diagnosis of the members of the Shigella group, plating on several media is an established routine. A variety of media was plated from the Bangxang and Eliot fluid, and the outcome evaluated.

The new plate of Wilson and Blair was used simultaneously with the plates of MacConkey; Holt, Harris, and Teague; Leifson; Endo; Wilson, Blair, Hajna and Perry; and selenite enrichment with consecutive plating on one of Leifson's plates, in the examination of proctoscopic specimens.

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Shortly after Wilson and Blair[2] described their new medium for the cultivation of *Shigella paradysenteriae*, it was praised by Thomas and Hulme.[3] On this plate, *Shigella paradysenteriae* grows in pink or colorless colonies. Strong lactose fermenters form a yellow growth. Escherichiae, Aerobacters, Protei, and Pseudomonades are said to be retarded.

It was found that the rosolic acid and the potassium tellurite used in the new medium of Wilson and Blair have to be standardized against known strains. The pH of the medium must be carefully observed. The alteration of the pH by the addition of the iron citrate, rosolic acid, and tellurite must be corrected to the final optimum of 7.6 to 7.8. At this pH, not only Flexner strains, but also Sonne strains grow on this medium very well. Heavy inocula may be used without courting the danger of overgrowth. While some fast growing strains of Proteus and Pseudomonas render the interpretation even of such selective plates as those of Leifson difficult, Wilson and Blair's medium is not disturbed by spreaders.

MacConkey's medium was prepared with the addition of 0.3 Gm. chloral hydrate per liter.[4] In this form, the spreading of most Proteus strains is checked. Addition of 3 c.c. of a 0.4 per cent solution of bromthymol blue per liter of medium rendered the medium more legible.

The media of Holt, Harris, and Teague, Leifson's desoxycholate plate, and Endo's medium were prepared according to the prescriptions of the American Standard Methods.[5] Leifson's desoxycholate-citrate plate was purchased on the open market. Endo's medium was omitted after it failed in 60 per cent of the first series of examinations.

Over eight hundred proctoscopic specimens were examined one to six hours after they were taken. Fifty-one specimens harbored *Shigella paradysenteriae* (Flexner). Twenty-two revealed *Shigella sonnei*, and one contained members of both groups. Many positive specimens were taken from the same individual over varying time intervals.

Twenty-five specimens gave a positive result on all four plates (desoxycholate-citrate, MacConkey, Wilson-Blair, and Teague).

Ten specimens were positive on desoxycholate-citrate, MacConkey, and Wilson-Blair medium, but negative on Teague.

Five specimens were positive on desoxycholate-citrate, Wilson-Blair, and Teague, but negative on MacConkey.

Seven specimens were positive on desoxycholate-citrate, MacConkey, and Teague, but negative on Wilson-Blair.

Two specimens were positive on MacConkey, Wilson-Blair, and Teague, but negative on desoxycholate-citrate.

Two specimens were positive only on desoxycholate-citrate and MacConkey.

Eight specimens were positive only on desoxycholate-citrate and Wilson-Blair.

Eight specimens were positive only on MacConkey and Teague.

Three specimens were positive only on desoxycholate-citrate.

One specimen was positive only on MacConkey.

Two specimens were positive only on Wilson-Blair.

One specimen was positive only on Teague.

Summarizing these results, from the seventy-four positive specimens:

Sixty were positive on the desoxycholate-citrate plate	82 per cent.
Fifty-eight were positive on MacConkey's medium	78 per cent.
Fifty-six were positive on Wilson-Blair's new plate	70 per cent.
Forty-eight were positive on Teague's medium	65 per cent.

All three forms of colonies (S, R, and G) appeared only on the plate of MacConkey, on Leifson's desoxycholate medium, and to a smaller extent on Teague's plate. The desoxycholate-citrate medium does not permit the growth of all forms, an observation made already by Ruys.[6] Wilson and Blair's plate showed the same characteristics. The advantage of a "heavier" inoculum on selective media is therefore equilibrated by the advantage of "all colony forms growing" on nonselective plates.

Bangxang and Eliot's preserving fluid was effective in our cases when, between the taking of the specimen and the plating, some hours had elapsed.

It should be added that the procedure described revealed not only mannitol-fermenting Shigellae, but in two cases, *Eberthella typhi*, and in two other cases, *Salmonella suipestifer*.

SUMMARY

It seems that the only way to obtain a high number of positive results is to use several selective and nonselective plates simultaneously. The preserving fluid of Bangxang and Eliot is recommended. From the selective media those of Leifson, and of Wilson and Blair, from the nonselective plates those of MacConkey, and of Teague proved to be satisfactory. Wilson and Blair's new plate is not superior to other media. It is easy to prepare, it is selective, and it deserves therefore to be added to the series of routine plates.

I wish to thank Dr. H. J. Shaughnessy, Chief, Division of Laboratories, Department of Public Health, Chicago, Ill., and his assistant, Miss F. Friewer, Chief of the Typhoid Section of the Central Laboratory of the Department of Public Health, Chicago, Ill., for constant advice and helpful suggestions in this work.

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A METHOD FOR THE DETERMINATION OF STAPHYLOCOCCAL ANTITOXIN AND ANTICAPSULAR AGGLUTININ USING CAPILLARY BLOOD*

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DURING a study of staphylococcal antitoxin and anticapsular agglutinin in the blood of mothers and their infants, the difficulty of obtaining sufficiently large samples of venous blood from the infants prompted us to devise a method that would require only the small amounts of capillary blood obtainable by heel puncture. The method described herewith has proved to be highly satisfactory.

PREPARATION OF SAMPLE

Exactly 0.05 c.c. of capillary blood is drawn into a glass pipette previously rinsed with a 0.2 per cent solution of potassium oxalate in saline. The blood is introduced into a 10 mm. glass tube containing 0.45 c.c. of the oxalate solution and is shaken vigorously to avoid clotting. After centrifuging for a few minutes, 0.35 c.c. of the supernatant can be removed for the two determinations.

A. DETERMINATION OF ANTITOXIN

For titration of antitoxin, 0.25 c.c. of the supernatant is diluted in series, using 10 mm. test tubes containing 0.25 c.c. of physiologic saline (0.85 per cent NaCl). To each tube is added 0.25 c.c. of a 1:4 dilution of the staphylococcal toxin (hemolysin), as used for the routine titration of venous serum antitoxin, and the mixture is placed in a water bath at 37° C. for fifteen minutes. Finally, 0.5 c.c. of a 1 per cent suspension of fresh, thrice-washed rabbit erythrocytes is added to each tube, bringing the total volume to 1 c.c. The tubes are again placed in the water bath at 37° C. for an hour and then left in an icebox at 4° C. overnight. The following day the test mixtures are kept at room temperature for ten minutes before the presence of hemolysis is observed by gently rotating each tube in the rack. The hemoglobin always rises from the bottom of the tube before the unhemolyzed cells. In a dilution series the last tube showing no hemolysis represents the end point.

For each group of microdeterminations two controls were always used: (1) titration of a standard staphylococcal antitoxin by the routine method; (2) simultaneous titration of one subject's antitoxin by the routine, venous serum method and the micromethod. The standard antitoxin, a commercial preparation obtained from The Lederle Company, Pearl River, N. Y., was adjusted by dilution with saline so that 1 c.c. contained exactly one international unit of staphylococcal antitoxin. This preparation was stored in a sealed flask at 4° C. For the routine titration of antitoxin in venous serum, the method of

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Parish et al.[1] was used. The staphylococcal toxin was prepared in a manner similar to that of Dolman.[2] Complete details of these two procedures are presented elsewhere.[3]

A sample group of titrations is shown in Table I. By including the titration of a standard antitoxin, the use of any convenient strength (X) of toxin is possible, and the antitoxic titer of venous serum for Subject A can readily be expressed as international units. The 1:4 (X/4) dilution of toxin used in the micromethod was obtained empirically so that the end point for this method would be at the same tube as that for the routine method. For Subject A the end point for both methods is at Tube 4. It must be emphasized that the actual amount of serum in each dilution tube of the micromethod is not the same as that for the corresponding tube of the routine method. If one can standardize the micromethod so that the values obtained by it give a constant ratio with those of the routine method, however, the amount of antitoxin present in the sample of capillary blood can be expressed in terms of international units per c.c. of serum. This is indicated in Table I by the values in parentheses.

TABLE I

SAMPLE TITRATION OF STAPHYLOCOCCAL ANTITOXIN BY ROUTINE AND MICRO-METHODS

SPECIMENS TESTED	METHOD	TITRATION TUBES 1 2 3 4 5 6 7 8	INTERNATIONAL UNITS OF ANTITOXIN/C.C. SERUM	STRENGTH OF TOXIN USED
Standard antitoxin (1 int. unit/c.c.)	Routine	O O O O O H H H	1.0 (known)	X
Venous serum of Subject A	Routine	O O O O H H H H	0.5	X
Capillary blood of A	Micro	O O O O H H H H	(0.5)	X/4
Capillary blood of B	Micro	O O O O O H H H	(1.0)	X/4
Capillary blood of C	Micro	O O H H H H H H	(0.13)	X/4
Capillary blood of D	Micro	O O O O O O H H	(2.0)	X/4

H = Hemolysis of rabbit R.B.C.
O = No hemolysis of rabbit R.B.C.
X = Any convenient, freshly prepared dilution of the concentrated staphylococcal toxin.
( ) = Explained in text.

The simultaneous determination of antitoxin by both methods in a group of twenty-six subjects indicates that the micromethod is well standardized and reliable. The results are compared in Table II. In twenty-two of the twenty-eight sets of simultaneous determinations, the end point by the two methods was exactly the same, and in four more the difference was only a single tube. This is within the probable error of any duplicate neutralization titration. Poor agreement of the two methods was obtained in only two instances. One seems justified, therefore, in concluding that the method of measuring staphylococcal antitoxin by using capillary blood will yield results comparable to those of the routine method requiring venous blood in most instances.

B. DETERMINATION OF STAPHYLOCOCCAL ANTICAPSULAR AGGLUTININ

The agglutination tests carried out in the present study followed essentially the method described by Lyons.[4] A hemolytic toxin-producing *Staphylococcus aureus* isolated from a case of acute osteomyelitis was used.

A shallow layer of Douglas's broth containing 0.05 per cent of glucose was inoculated with 0.1 c.c. of a twelve-hour culture of the organism for each 5 c.c.

of broth. After three hours' growth at 37° C., the culture was killed by slowly heating to 100° C., centrifuged and resuspended in saline to 1/20 of its original volume. This suspension was used in the agglutination.

For agglutinin titration 0.1 c.c. of the clear supernatant from the capillary blood sample was diluted with 0.7 c.c. of saline. Further dilutions were made in series by transferring 0.4 c.c. of the preceding dilution to 0.4 c.c. of saline in the next tube.

TABLE II

SIMULTANEOUS DETERMINATION OF STAPHYLOCOCCAL ANTITOXIN BY ROUTINE METHOD AND MICROMETHOD

SUBJECT	END-POINT OF ROUTINE METHOD	END-POINT OF MICROMETHOD	ERROR
	Tube No.	Tube No.	
G	7	7	0
P	4	4	0
Be	5	5	0
W	2	2	0
de P	7	7	0
Do	7	7	0
H	3	4	+1 tube
Ba	2	2	0
Da	3	4	+1 tube
Li	5	5	0
	(duplicate determination)	5	
Li	5	5	0
K	5	5	0
Mo	1	1	0
La	2	2	0
Ma	3	5	+2 tubes
Mi	3	3	0
I	1	2	+1 tube
O	3	3	0
A	2	<1	−2 tubes
Ho	2	2	0
Lib	3	4	+1 tube
Oc	3	3*	0
Ca	4	4*	0
Za	5	5*	0
Mor	5	5*	0
Ba	5	5*	0
An	6	6*	0

*These tests were performed with toxin which was six months old and it was necessary to allow the tubes to stand at room temperature for one to two hours after removing them from the icebox before satisfactory hemolysis could be observed in the micromethod.

One drop of the suspension of staphylococci was mixed with a loopful of the serum dilution on a clear glass slide. A control test of the organisms and saline was always made on the same slide. The test mixtures were gently rotated for five minutes and then read. The photographic charts of Pauli and Coburn[5] served as standards for reading the degree of agglutination.

The accuracy of the method using capillary blood in the titration of the anticapsular agglutinin was determined by the simultaneous assay of venous blood serum and capillary blood. The results of seventeen parallel determinations (Table III) showed exact agreement in eleven cases (65 per cent). In the remaining six cases (35 per cent), the variation was less than one serum dilution interval.

The values used for the micromethod in Table III are based on the assumption that the supernatant of the centrifuged capillary blood represents a 1:10

TABLE III

THE SIMULTANEOUS DETERMINATION OF STAPHYLOCOCCAL ANTICAPSULAR AGGLUTININ BY ROUTINE METHOD AND MICROMETHOD

SUBJECT	SERUM FROM VENOUS BLOOD		SERUM FROM CAPILLARY BLOOD	
	COMPLETE AGGLUTINATION	PARTIAL AGGLUTINATION	COMPLETE AGGLUTINATION	PARTIAL AGGLUTINATION
J. A. L.	640	1280	640	1280
C. P. K.	640	1280	640	1280
O.	640	–	320	640
C.	640	1280	640	1280
Z.	640	1280	640	1280
J. A. L.	1280	–	640	1280
C. P. K.	640	1280	320	640
W. L. B.	640	–	320	640
A.	640	1280	640	1280
J. A. L.	640	–	640	1280
C. P. K.	1280	–	640	1280
W.	640	–	640	–
J. A. L.	640	–	640	–
B.	160	320	160	–
Mc	320	640	320	640
S.	320	640	320	640
D.	160	320	320	–

Complete agglutination = 4+
Partial agglutination = 1 to 3+
No agglutination = –

dilution of the capillary serum. This dilution is probably not 1:10, but ranges from 1:10 to 1:20, depending on the hematocrit value of the capillary blood. The results for the venous and capillary sera in Table III, however, agree more closely if the original dilution of the capillary serum is assumed to be 1:10 rather than 1:20. We have no definite explanation for this finding, but it may be due to a greater concentration of agglutinin in capillary as compared with venous serum. This notion is supported by the results obtained by calculating the ratio of staphylococcal toxin to antitoxin in the micromethod on the basis of that observed for the routine (venous serum) method. If this be done, one finds that capillary serum actually neutralizes from 2½ to 5 times the expected amount of toxin, depending on the dilution of the serum (from 1:10 to 1:20) in the sample of capillary blood. Thus, the staphylococcal antitoxin likewise appears to be more concentrated in capillary than in venous serum.

CONCLUSIONS

A method is described for the simultaneous titration of staphylococcal antitoxin and anticapsular agglutinin using 0.05 c.c. of capillary blood. A comparison of this method with the standard procedure for the titration of each antibody in venous serum shows an agreement satisfactory for serologic methods.

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CHEMICAL

THE DETERMINATION OF THIAMIN IN BLOOD*

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IT IS now generally agreed that the determination of thiamin in blood and urine is a valuable aid in the diagnosis of thiamin deficiency. Fairly accurate chemical methods have been described for the determination of thiamin in urine. It has been shown that the daily excretion of the vitamin by the kidney is related to the intake and absorption of dietary thiamin and that the determination is of considerable value, provided the subject continues his customary diet during the 24-hour period of urine collection.[1, 2] On the other hand, because of technical difficulties, chemical methods have not been successfully applied to blood. The most reliable results have been obtained by means of the cocarboxylase methods of Ochoa and Peters[3] and Goodhart and Sinclair[4, 5, 6] and the yeast fermentation method of Schultz, Atkin, and Frey.[7, 8] The first is specific for diphosphothiamin, or cocarboxylase; the second is not. Both are subject to many variations, and the techniques must therefore be carefully controlled. The fermentation method can be expected to yield higher results, since it determines the total thiamin content. The difference should be slight, however, because blood contains very little free thiamin. By using these procedures, Goodhart has shown that the whole blood of human subjects contains from 3 to 9 micrograms of total thiamin per 100 c.c. Thus, by means of the cocarboxylase method, this author found from 2.9 to 9.4, with an average of 5.4 micrograms per 100 c.c. in the blood of 50 children varying in age from 4 to 15 years.[9] The blood of 26 healthy adults contained from 3.2 to 8.7, with an average of 5.0 micrograms per 100 c.c.[4] When the fermentation method was used, the blood of 45 normal subjects contained from 3.1 to 9.2, with an average of 5.39 micrograms.[8] Our experience, in agreement with that of others, has shown that the total thiamin content of blood does not fluctuate as widely as the urinary excretion: it is not as rapidly affected by the dietary intake of thiamin. Yet a very definite correlation is noted between the concentration of total thiamin in the blood and the incidence of deficiency symptoms. Since a high percentage of subjects with polyneuropathy have blood values below 3 micrograms per 100 c.c., Goodhart has suggested that a concentration below this level indicates a state of deficiency.

The chemical procedure described by us, besides having improvements in technique, embodies many of the features described in the numerous modifications of the Jansen thiochrome method.[10-20] The principal difficulty in the analysis

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of blood is in the precipitation of the large amount of soluble protein. This is readily accomplished by means of metaphosphoric acid, which always yields solutions which rapidly pass through the zeolite adsorbent and allow an excellent recovery of added thiamin. With a similar method, using trichloroacetic acid, Hennessy and Cerecedo[16] found from 9 to 12 micrograms per 100 c.c. of thiamin in blood. Our results, obtained by the analysis of 75 samples from 36 subjects, varied from 3.0 to 11.2, with an average of 5.70 micrograms per 100 c.c. Our data, therefore, agree substantially with those of Goodhart.

PROCEDURE

Reagents

Approximately N HCl and M $NaHCO_3$ Solutions

Metaphosphoric Acid.—A 10 per cent solution is prepared at frequent intervals, preferably each day. Since the acid deteriorates rapidly at room temperature, it should be dissolved in cold water in the refrigerator and the solution kept there when not in use.

Phosphatase.—A 10 per cent solution of taka diastase[16] or clarase[19] is prepared each day. Only the clear supernatant solution or filtrate is used.

Ion Exchange Adsorbents.—Artificial zeolites, such as Decalso,*[16] Permutit,*[18] or Crystallite† may be used. We prefer a mixture of equal parts of zeolites of 30- to 50-mesh and 50- to 80-mesh size. Columns prepared from such a mixture are not readily clogged with colloidal material and solid particles which sometimes are present in the final solution. One kilo of zeolite is covered and thoroughly mixed with 3 to 4 changes of an acidified (2 c.c. of concentrated HCl per liter) 25 per cent solution of NaCl. If ordinary table salt is used, the solution should be filtered before the addition of the acid. The supernatant solution is removed by decantation each time. The zeolite is then washed 5 or more times by decantation with acidified distilled water (1 c.c. of glacial acetic acid per liter). This washing removes the finer material as well as the excess of salt. The preparation is then transferred to a large Buchner funnel, is well washed with distilled water, and finally dried at room temperature in a shallow pan. In order to have the material in its maximum expanded state, a small portion is kept moistened in a widemouthed bottle.

NaCl Solution.—Each liter contains 250 Gm. of C.P. NaCl and 2 c.c. of concentrated HCl. Solutions prepared from table salt contain materials which slightly reduce the yield of thiochrome from thiamin. If table salt is used, the solution should be filtered before acidification with HCl.

Oxidizing Reagent.[11, 13, 18]—A mixture consisting of 9 parts of 10 *N* NaOH and 1 part of a 1 per cent solution of $K_3Fe(CN)_6$ is freshly prepared each day.

Iso-butyl Alcohol.[13]—The fluorescence without oxidation should be not greater than that of distilled water. Higher fluorescing batches may be used in the analysis of urine, but *they should never be used in the analysis of blood.* Should the alcohol contain much fluorescing material, it is acidified with con-

*This is obtained from the Permutit Company, New York City, New York.

†Crystallite, when prepared with the recommended very acid salt solution, does not adsorb the vitamin as well as Decalso. It may be obtained from the International Filter Company, 325 W. 25th Place, Chicago, Illinois.

centrated HCl, dehydrated with Na_2SO_4, and redistilled. Butyl alcohol may also be used.[12, 19]

The used alcohol is poured into a bottle which contains an excess of HCl. The acidified alcohol is distilled. Much water separates from the fraction which distills up to 100° C. All fractions contain water, which is removed with anhydrous Na_2SO_4 before redistillation. Calcium oxide is not used because traces of alkali in the distillate catalyze the formation of fluorescing substances.

Quinine Sulfate Standard.[20]—One hundred mg. of quinine sulfate, U.S.P., are transferred to a volumetric flask of 1 liter capacity. One hundred c.c. of approximately *N* H_2SO_4 are added to about 800 c.c. of solution and the volume is brought to the mark. The intermediate standard is prepared by diluting 25 c.c. of the stock standard to 100 c.c. with water and enough *N* H_2SO_4 to bring the final acidity to 0.1 *N*. The solutions are stable for at least one year if kept in the refrigerator in an amber-colored bottle. To prepare the working standard, exactly 5 c.c. of intermediate standard are diluted to 500 c.c. with water and H_2SO_4 as before. The solution should be prepared frequently. It should be protected from light and stored in the refrigerator. The fluorescence of 10 c.c. of this solution in a cuvette is approximately the same as that given by 10 c.c. of extract obtained after oxidation of 1 microgram of thiamin to thiochrome.

Thiamin Standard.—Exactly 50 mg. of anhydrous thiamin hydrochloride (dried several weeks over H_2SO_4) are dissolved in a 25 per cent solution of ethyl alcohol in approximately 0.01 *N* HCl. When brought to a volume of 500 c.c. with this solution, each c.c. contains 100 micrograms. This stock solution, if kept cold, is stable for at least six months. To prepare the intermediate standard, 25 c.c. of the stock solution are pipetted into a 250 c.c. volumetric flask which contains 100 c.c. of water and 25 c.c. of 0.1 *N* HCl. The volume is then brought to the mark. The working standard is prepared when needed by diluting 5 c.c. of the intermediate standard to a volume of 250 c.c. with 0.01 *N* HCl. Five c.c. contain 1 microgram of thiamin.

ANALYSIS OF WHOLE BLOOD

Collection of Sample.—Blood is collected with a syringe and prevented from clotting with a minimum of oxalate. *It is then stored in the refrigerator and the analysis is made within a few hours of collection.* These precautions are necessary, since the thiamin content changes gradually even on storage of the sample in the cold.

Digestion With Phosphatase.—Five c.c. of blood are mixed with 25 c.c. of water in a large heavy-walled test tube such as is used in the determination of nonprotein nitrogen. Two c.c. of *N* HCl are added, and the contents are mixed by means of a long thin footed glass rod.* The mixture is heated ten minutes with frequent stirring in a boiling water bath. It is then cooled, after which 1 drop of caprylic alcohol and 2 c.c. of *M* $NaHCO_3$ solution are added. The latter should be run in slowly and with stirring. This volume of solution is sufficient to bring the reaction to pH 4.5 to 5.5. 2 c.c. of phosphatase solution is now

*A J-shaped rod, with a round bend fitting the bottom of the tube is recommended. With such a rod the contents of the tube can be violently agitated by rolling the rod back and forth between the thumb and index finger. The rod should remain in the tube until the protein have been precipitated.

added. The tube is kept 1 to 1.5 hours in a water bath at 40 to 45° C. during which time the contents are frequently mixed by stirring.*

Identical results are obtained with the following slightly modified procedure: 0.5 c.c. of *N* HCl is added to the laked blood, which is followed by incubation with 0.2 to 0.25 Gm. of phosphatase for one hour. The mixture is then further acidified with 1.5 c.c. of *N* HCl, after which it is heated ten minutes in a boiling water bath with frequent stirring, and then cooled.

A blank is prepared by adding the same reagents in the order indicated. If the alternate procedure is used, the phosphatase and 1 drop of *N* HCl are added to 25 c.c. of water. The mixture is incubated at 40 to 45° C. This blank, rather than the salt blank, is necessary to correct for the small amount of thiamin present in the phosphatase.

Precipitation of Proteins.—Ten c.c. of HPO_3 solution are next added. The volume is adjusted to the 50 c.c. mark and the contents are well mixed. The mixture is centrifugated fifteen minutes. The precipitate may contain some adsorbed thiamin which may be recovered by re-extraction. It is therefore emulsified with 2.5 c.c. of HPO_3 solution and the volume is again brought to 50 c.c.

Adsorption and Elution.—The necessary adsorption columns are prepared some time before the analysis and kept in a beaker of water until ready for use. A plug of glass wool (Pyrex No. 719) is pushed to the bottom of the Hennessy tube;† the tube is filled with water, and small quantities of moist zeolite are added until an excess, forming a layer several mm. deep, is present in the bell above the column. The adsorbent is more uniformly packed when floated into place in this manner. The excess of zeolite in the bell prevents clogging of the column by solid or colloidal material. Furthermore, it prevents access of air into the column, provided the drained column is not jarred. Just before use, water is drawn from the beaker upward through the column into the bell. The column is then placed in the rack† and allowed to drain. This assures a rapidly draining column which is free from air.

Brom phenol blue indicator is now added to the combined extracts contained in a 150 c.c. extraction flask, and the reaction is adjusted to 3.0 to 3.5 by means of *N* NaOH (or M $NaHCO_3$). The extract is then passed through the column. After passage of all of the extract, the flask is rinsed with several successive portions of about 5 c.c. each of distilled water, and the washings are sent through the column. The fluid is allowed to drain completely after each of the washings. Small portions of acidified 25 per cent NaCl solution are now added until exactly 25 c.c. of eluate have been collected in a calibrated test tube such as is used in the determination of nonprotein nitrogen.

*More or less acid, and correspondingly more or less bicarbonate solution, are necessary when tissues, feces, foods, or other materials are analyzed. The volume of acid and the length of heating in the water bath are different for each class of substance. In every case, the optimum conditions for maximum yield and recovery of added thiamin must be determined. When this is done, the procedure given by us can be applied generally to all materials. In the case of substances rich in thiamin, the contents of the tube are transferred after the digestion to a 250 c.c. volumetric flask; 15 c.c. of HPO_3 solution are added, and a suitable aliquot is analyzed as described below.

†The tube of Hennessy with the long (125mm.) stem is preferred to others which are now being sold. With the coarser mixture of zeolite recommended by us, more uniform results are obtained when the longer stemmed columns are used.

‡The rack consists of a 2″ x 2″ board which is mounted approximately 10 inches above the table. Holes ⅜″ in diameter are spaced 4 inches apart.

Melnick and Field, Hennessy and Cerecedo, and others recommend that the solution be heated and then passed through the column; the elution is carried out with hot 25 per cent solution of KCl. Heat is not necessary when a rapidly draining column, prepared as described by us, is used. *The adsorption of thiamin is equally complete from hot or cold solutions, and the vitamin is recovered just as well with cold 25 per cent solution of NaCl.*

The removal of ions by zeolites is most efficient at neutral or slightly alkaline reaction.[21] At this reaction the recovery of thiamin may be smaller than at pH 3, however, especially if the solution is heated. This is due perhaps to the sensitivity of the vitamin to alkali. For this reason the neutralization with strong NaOH must be carried out carefully.

Oxidation to Thiochrome.—Two aliquots of 10 c.c. each of the eluate are transferred to Hennessy reaction vessels of 50 c.c. capacity. Five c.c. of oxidation mixture are rapidly added, with mixing of the contents. This is followed immediately with 13 c.c. of iso-butyl alcohol. The contents are then shaken ninety seconds. Four oxidations are carried out in rapid succession. After centrifugation for a few minutes at low speed, the lower layer is drawn off, and 3 to 4 small scoopfuls (about 0.2 Gm. each) of anhydrous sodium sulfate are added. *The solvent must not be shaken with the salt, as this may produce an emulsion.* When added in this manner, the first scoopful of sulfate entraps the remaining solution at the bottom of the vessel. This is covered by successive layers of less hydrated sulfate which can then remove the water more effectively from the alcoholic phase. The extract becomes crystal-clear on standing one-half minute or so. If not clear, the mixture is very gently rotated, and 1 or 2 scoopfuls of the sulfate are added and the mixture is again centrifuged for one minute. Without further waiting, the fluorescence is then determined.

The blank is oxidized and extracted in a similar manner.

The thiamin content is obtained from a calibration curve representing the increase of fluorescence after the addition of 0.2, 0.4, 0.6, 0.8, or 1.0 microgram of thiamin to blood samples which have been analyzed by the procedure.

It will be noted that the oxidation is carried out at a considerably higher alkalinity than that used by previous workers, although a trend toward the use of larger quantities of alkali is noted in recent papers.[19, 22] More consistent results are obtained at this high alkalinity, due perhaps to the greater ionic concentration which favors the extraction of thiochrome by iso-butyl alcohol.

EXPERIMENTAL

Results of analysis of blood taken from 7 women and 29 men, a total of 36 subjects, are shown in Table I. The samples were collected from 10 to 12 in the morning and from 2 to 5 o'clock in the afternoon. They were thus taken from two to five hours after a meal. None of the samples were taken under basal conditions. These conditions were chosen because they were most convenient to the majority of subjects. They represent also the conditions under which blood is most conveniently taken from large groups of subjects in a nutritional study. In the order of numbers, the subjects consisted of research workers, students, internes, physicians, and janitors. None had received any vitamin preparations within the preceding month. All were in apparent good health, and all were receiving adequate diets. The blood of the women contained from

3.0 to 9.2, and that of the men from 3.8 to 11.2 micrograms per 100 c.c. The arithmetic mean of the results from the women was 5.60, and from the men, 5.73 micrograms per 100 c.c. The mean of all of the determinations was 5.70 micrograms per 100 c.c.

TABLE I

THIAMIN CONTENT OF WHOLE BLOOD OF HUMAN SUBJECTS

	WOMEN	MEN
Subjects	7	29
Determinations	18	57
Range, micrograms per 100 c.c.	3.0 to 9.2	3.8 to 11.2
Arithmetic mean, micrograms per 100 c.c.	5.60	5.73

Diet can affect the thiamin content of the blood. The level is fairly stable on a constant diet. Thus, the blood of subject B. J. contained 6.1 micrograms per 100 c.c. at the beginning of an experiment in which the subject received a high caloric diet which contained from 1500 to 1900 micrograms of thiamin per day. The thiamin levels, determined every other day during the second and third weeks of the experiment, were 4.5, 4.3, 4.5, 5.5, 5.0, 4.5, and 4.3 micrograms per 100 c.c. On a diet containing approximately 2500 micrograms of thiamin per day, the blood of subject T. F. taken at monthly intervals contained 7.0, 9.9, and 9.9 micrograms of thiamin per 100 c.c. After two weeks, however, during which the subject received a diet containing about 300 micrograms of thiamin per day, the blood level fell to 2.8 micrograms per 100 c.c.

The following data are representative of the results which may be obtained from apparently healthy human subjects. Samples were obtained at approximately monthly intervals. The results are expressed in micrograms per 100 c.c.: K., 5.8, 6.0, and 4.9; J. H., 5.0, 5.8, and 3.9; E. A., 5.1, 8.0, and 5.1; E. G., 4.4, 7.5, 6.1, and 6.8; H., 9.2, 5.4, 3.0, and 4.1.

SUMMARY AND CONCLUSIONS

A procedure is described for the determination of thiamin in 5 c.c. of oxalated whole blood by the thiochrome method.

The blood of 36 human subjects in apparent good health and receiving an adequate diet contained from 3.0 to 11.2, with an average of 5.70 micrograms per 100 c.c. These results agree substantially with those obtained by previous workers with the cocarboxylase and fermentation methods.

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A METHOD FOR DETERMINING CEREBROSPINAL FLUID PROTEIN BY THE PHOTOELECTRIC COLORIMETER*

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THE estimation of the protein content of cerebrospinal fluid has become an increasingly important part of laboratory routine. The ideal method should combine accuracy with speed of operation and the use of minimal quantities of spinal fluid. The method of Denis and Ayer, as modified by Ayer, Dailey, and Fremont-Smith,[1] has proved very satisfactory, but the accuracy of the determination depends upon the ability of the technician to match turbidities in the ordinary colorimeter.

The photoelectric colorimeter has eliminated this personal factor, and turbidimetric methods would seem to be ideally suited to the instrument. Looney and Walsh[2] described such a method, using 2 c.c. of spinal fluid. Ikeda and Hanson[3] presented a similar method for further trial and evaluation. The latter method requires the use of 3 c.c. of fluid. Neither of these papers mentions the error introduced by color in some pathologic fluids.

The following method is a modification of that presented by Ayer, Dailey, and Fremont-Smith[1] for the standard colorimeter. One c.c. of fluid is used instead of the original 0.6 c.c. in order to simplify pipetting.

METHOD

One c.c. of cerebrospinal fluid is accurately measured into a test tube and 1 c.c. of 5 per cent sulphosalicylic acid is added. The tube is inverted or rotated gently to ensure thorough mixing and is allowed to stand at least five minutes before reading. The tube is then inverted again and the mixture is transferred to a small colorimeter tube after the latter has been rinsed with 2 to 3 drops of the mixture. The tube is placed in the colorimeter and the deflection is read on the galvanometer.

The center setting is determined with a tube containing 1 c.c. of distilled water and 1 c.c. of 5 per cent sulphosalicylic acid and can be used for all fluids that are clear and colorless. We have found a preliminary "warming up" period of five minutes to be sufficient for the colorimeter, provided care is taken to adjust the center setting before each reading.

All fluids which are not clear and colorless have been found to give false high results due to the added absorption of light by color. This can be compensated for by adding 1 c.c. of water to 1 c.c. of the fluid (in whatever dilution has been used for precipitation) and using this as a blank to determine the center setting. Preliminary detection of color depends upon the eye of the technician, but where there is the slightest doubt a blank reading should be made in this way. Such readings may show a deflection of as much as ten divisions

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on the galvanometer above the center setting (approximately 65 on a galvanometer scale divided in 100 equal parts) obtained with the water and acid blank. In contrast to this, a series of cerebrospinal fluid plus water blanks was run on 15 "clear and colorless" fluids. Fourteen of these varied only one-half of a division, and the fifteenth, three-fourths of a division, from the deflection of the water plus acid blank.

Protein concentrations from 12 to 110 mg. per 100 c.c. (17 to 76 per cent light absorption) can be estimated directly. Fluids containing higher concentrations must be diluted so that the reading falls within those limits.

APPARATUS

The apparatus consists of a miniature colorimeter of the Evelyn type[4] built to accommodate small Pyrex test tubes (8 × 75 mm.). These tubes were carefully tested for uniformity and only those conforming to the standard set by the Rubicon Company were used.

The miniature attachment for the photoelectric colorimeter is constructed as follows: Light from a 6.3 volt lamp passes through a built-in filter (Rubicon 520) and then through the solution by way of a slit 0.25 inch wide and 0.75 inch high. The test tube is so aligned that the bottom of the slit is just above the rounded portion of the tube. A removable, lightproof cap is necessary to prevent stray light from reaching the photocell via the top of the tube. The light intensity is recorded by means of a General Electric blocking layer photocell.

The instrument is included in the case of the standard Evelyn colorimeter and makes use of the same light control circuit and galvanometer. A suitable switch allows the convenient use of either instrument.

CALIBRATION

A standard protein solution was prepared according to the method of Ayer, Dailey, and Fremont-Smith.[1] Dilutions were made to give eleven standard solutions ranging in concentration from 12 to 110 mg. per 100 c.c. The protein content of each was determined in duplicate by the micro-Kjeldahl method and also the light absorption of each, after precipitation with sulphosalicylic acid, was measured in the photoelectric colorimeter. Using the actual readings on the galvanometer scale as ordinate and the concentration of protein (as determined by micro-Kjeldahl) as abscissa, points were established on graph paper and a curve drawn. This curve has been used to estimate the protein in all fluids of unknown concentration.

Duplicate determinations of protein were made on numerous samples of cerebrospinal fluid both by the photoelectric method and by micro-Kjeldahl. As noted by Ayer, Dailey, and Fremont-Smith,[1] difficulty was encountered in obtaining satisfactory checks with the micro-Kjeldahl when the nonprotein nitrogen was subtracted from the total nitrogen and the result multiplied by the factor 6.25. More consistent results were obtained by precipitating the protein with trichloracetic acid and determining the nitrogen content of the precipitate directly. This method has been compared with the direct gravimetric method by Guillaumin et al.[5] and has been found to check closely with it. It has the added advantage of requiring only two micro-Kjeldahl determinations instead of four for duplicate estimations.

TABLE I

TOTAL PROTEIN, MG. PER 100 C.C.

PHOTOELECTRIC	MICRO-KJELDAHL (TOTAL N. MINUS N.P.N.)
14.3 & 14.5	16.9 & 14.4
15.6 & 15.8	21.9 & 22.5
20.2 & 20.2	21.2 & 24.4
20.5 & 20.8	18.1 & 19.3
22.3 & 21.8	31.3 & 30.0
27.5 & 27.5	31.3 & 32.5
39.5 & 39.5	43.1 & 40.6
48.0 & 48.7	50.6 & 46.9
49.2 & 48.7	51.3 & 45.0
55.0 & 55.3	54.4 & 53.1
57.0 & 57.3*	63.8 & 64.4
57.8 & 57.0	60.6 & 55.6
63.2 & 64.2	58.1 & 63.1
72.8 & 73.5	83.1 & 88.1
73.5 & 74.2	78.1 & 74.4
82.0 & 81.3	79.2 & 77.9
89.0 & 87.5	92.5 & 87.5
90.3 & 91.3	101.9 & 103.1

*Xanthochromic fluid—center setting adjusted.

TABLE II

TOTAL PROTEIN, MG. PER 100 C.C.

PHOTOELECTRIC	MICRO-KJELDAHL (PROTEIN NITROGEN)
12.5 & 12.3	12.5 & 9.8
14.7 & 14.9	17.5 & 17.9
19.5 & 19.7	24.1 & 20.1
21.3 & 21.1	20.1 & 20.7
27.8 & 27.8	28.4 & 25.2
28.7 & 28.5	31.5 & 32.6
39.4 & 39.2	36.6 & 37.0
44.3 & 44.0	45.5 & 45.5
50.7 & 50.7	47.7 & 47.3
54.2 *	56.4 & 56.0
59.3 & 59.7*	55.6 & 57.8
65.3 & 65.7*	60.4 & 62.1
68.2 & 69.2	63.8 & 69.1
98.0 & 99.6*	93.6 & 91.0
189.0 & 192.4	184.6 & 181.1
422.5 & 425.5*	428.7 & 446.3
6,480 & 6,430†	6,191 & 5,906

*Xanthochromic fluids—center setting adjusted.
†Cyst fluid—center setting adjusted.

RESULTS

All the figures obtained in the course of the experiment are presented in Tables I and II. It will be noted that the results of photoelectric determinations are in more close agreement with the micro-Kjeldahl results in Table II (protein nitrogen) than is the case in Table I (total nitrogen minus nonprotein nitrogen). Duplicate figures in the photoelectric method are remarkably consistent, and where the figures by micro-Kjeldahl determination were not in such close agreement the photoelectric results will be found to check fairly closely with one or the other of them. In only two instances (the second and the fourteenth) in Table II were the duplicate values for each method in close agreement while showing a distinct difference between the two methods. In one of these the

figures by photoelectric method were higher, and in the other they were lower than the Micro-Kjeldahl results.

The method is convenient and inexpensive. A single determination requires about ten minutes and even less time per determination if a number of samples are to be tested.

SUMMARY

1. A simple method is presented for the determination of cerebrospinal fluid protein with the photoelectric colorimeter.

2. A miniature attachment for the Evelyn photoelectric colorimeter is described which will accommodate small Pyrex test tubes. This permits the determination to be carried out on 1 c.c. of spinal fluid.

3. False high results may be obtained with colored specimens of spinal fluid. A method to rule out this source of error is described.

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DETERMINATION OF MANDELIC ACID IN URINE*

Precipitation as Cadmium Mandelate

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MANDELIC acid and salts of mandelic acid are employed in the treatment of urinary infections and after their administration the physician may find it desirable to follow the concentration of mandelic acid in the urine of the patient. In a clinical investigation[2] on the injection of solutions of sodium mandelate this laboratory was requested to supply the analytical data. The available analytical procedures for the determination of mandelic acid in urine were studied and found to be unreliable. A new gravimetric method was developed in which the mandelic acid in the urine is extracted with ether, converted to the sodium salt for extraction and concentration in an aqueous solution, and precipitated and weighed as cadmium mandelate. The method may be carried out

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fairly rapidly on a semimicro scale with 10 c.c. samples of urine, or it may be applied as a macro procedure with 100 c.c. samples. The concentration range of 0.1 to 2.0 Gm. of mandelic acid in 100 c.c. of sample was studied since this represents the normal range of concentrations encountered during mandelic acid therapy.

In 1909 Neubauer[8] determined the mandelic acid excreted by dogs after feeding experiments. The acid was extracted from the urine by ether and, after purification, was titrated with 0.1N alkali. Rosenheim,[11] who introduced mandelic acid therapy in 1935, determined mandelic acid in the urine of his patients by extracting with ether after acidifying the specimen with phosphoric acid and saturating with ammonium sulfate. The residue obtained by evaporating the ether was weighed as mandelic acid. McMahon[6] combined the Rosenheim extraction method with the titration method used by Neubauer. Montenbruck,[7] and also Garry and Smith,[3] determined the extracted *d* or *l* mandelic acid polarimetrically. This method is not applicable in human cases, however, since the inactive *dl* mandelic acid is usually administered, and it has been shown[7] that both forms of mandelic acid are excreted simultaneously. For the assay of medicinal preparations, Stevens and Hughes,[12] and also Rosen,[10] employed continuous extraction with ether followed by titration with standard alkali. Underwood[13] recommended extraction with a mixture of two parts chloroform and one part ether, followed by titration with standard alkali.

The published methods are subject to several errors. Reimers[9] indicated that care must be taken in evaporating the ether from the extract in order to avoid loss of mandelic acid by volatilization. McMahon reported the loss of an average of 6 per cent of mandelic acid in recovery experiments from water with samples ranging from 0.5 to 4.5 Gm., and 16 per cent loss in recovery from urine with samples ranging from 0.2 to 0.3 Gm. The loss was attributed to combination with other constituents of the urine. Berger,[1] using McMahon's method, reported a loss of about 10 per cent from 0.1 gram samples in recovery of mandelic acid from urine "through the combination with other urinary constituents or through inaccuracy of the method." He also reported a loss of 20 per cent in the recovery of mandelic acid added to breast milk.

DEVELOPMENT OF GRAVIMETRIC METHOD

A study of the methods reviewed above indicated a need for a more satisfactory procedure for the determination of mandelic acid in urine. In attempting to apply Rosenheim's method of extraction from 100 c.c. portions of urine, intractable emulsions were frequently encountered, but the use of the continuous method of extraction with ether recommended by Stevens and Hughes entirely overcame this difficulty. It was found necessary to acidify the urine rather strongly to insure the complete liberation of the mandelic acid. High acidity has been shown[4] to decrease the solubility of mandelic acid in water and would be expected to facilitate its extraction by ether. The extracted mandelic acid, however, was always found to be impure, and direct titration generally gave results which were high. It was found that pure mandelic acid can be nearly quantitatively precipitated by adding concentrated hydrochloric acid or

by saturating the solution with sodium chloride, but mandelic acid recovered from urine under these conditions crystallized only slowly and incompletely, presumably on account of the inhibiting action of the accompanying impurities.

Mandelic acid extracted from urine by means of ether was found to be promptly and quantitatively precipitated as the cadmium salt from aqueous solution, furnishing the basis for a satisfactory gravimetric method. McKenzie[5] prepared cadmium mandelate and listed its solubility as 0.1 Gm. in 100 c.c. of water at 16° C. Since McKenzie did not publish his analytical data, the present authors have determined the composition of cadmium mandelate precipitated under various conditions and have redetermined its solubility.

It was noted that the physical characteristics of the crystalline cadmium mandelate formed at room temperature changed appreciably when the solution with precipitate was heated on the steam bath. The cadmium mandelate was formed first as a dense crystalline precipitate which upon heating changed to a flocculent form. Pure cadmium mandelate is white, but when precipitated from urine it may be slightly colored. It was subsequently established that both forms of precipitate had the same composition after they were dried. Samples representing each of the two forms of precipitate were prepared and analyzed. Cadmium mandelate was precipitated by adding an excess of cadmium chloride solution (50 Gm. cadmium chloride in 100 c.c. water) to a slightly acidified aqueous solution of sodium mandelate, the conditions adopted for the analytical procedure described later. In one case the cadmium mandelate was precipitated at room temperature, after which it was heated on the steam bath until the physical change in the crystalline structure was noted. The precipitate was collected on a sintered glass funnel and washed with small portions of distilled water until free from chloride (Sample No. 1). A second sample of cadmium mandelate was prepared by precipitation from a previously cooled solution of sodium mandelate, and the solution and crystals were kept in an ice bath until the washing of the precipitate was completed (Sample No. 2). The two samples were dried in a vacuum desiccator and analyzed by simultaneous microcombustion for cadmium, carbon, and hydrogen. Cadmium was converted to the oxide during the course of the carbon and hydrogen combustion, and the percentage of cadmium was calculated from the residue of cadmium oxide remaining in the platinum boat. A complete combustion of the cadmium mandelate was easily obtained at a temperature well below the decomposition temperature of cadmium oxide, 900 to 1000° C. During the combustion the boat was not heated above a dull red heat. The results are shown in Table I. The values obtained for percentage composition indicated that $Cd(C_8H_7O_3)_2$ is the correct formula for both forms of precipitate. It is probable that the differences in physical characteristics are associated with hydrate formation.

Solubility determinations were made using portions of the purified cadmium mandelate samples described above, at 25° C., in water and in dilute acetic acid (the conditions recommended in the analytical procedure). Approximately 0.2 Gm. of cadmium mandelate was placed in a 200 c.c. Kjeldahl flask together with 100 c.c. of distilled water. The flask was fitted with a motor-driven glass stirrer and clamped in a constant temperature water bath. The contents of the flask were stirred slowly for three hours, at which time a sample of 15 c.c. was with-

drawn through a sintered glass filter stick into a calibrated pipette and transferred to a previously tared micro-evaporating dish. The water was cautiously evaporated on a steam bath, the dish and residue dried in a vacuum desiccator over phosphorus pentoxide and again weighed. The increase in weight was calculated to grams of cadmium mandelate per 100 c.c. of solution. A second portion was withdrawn three hours later to determine whether the equilibrium point had been attained. As a check on the results, a determination was made in which the original mixture was heated to 100° C. and then cooled to 25° C. Another determination was made in an acetic acid solution pH 4.11. The results of these determinations are shown in Table II. The average value for solubility, 0.08 Gm. in 100 c.c. of solution, is slightly lower than the value reported by McKenzie. The solubility was found to be only slightly greater in acetic acid than in water.

TABLE I

ANALYSIS OF CADMIUM MANDELATE

	Cd	C	H
	PER CENT	PER CENT	PER CENT
Theory $Cd(C_8H_7O_3)_2$	27.1	46.3	3.4
Sample No. 1	27.2	(47.5)	3.7
	27.3	46.3	3.7
	27.3	46.6	3.7
Sample No. 2	27.2	46.7	3.2
	27.1	46.3	(4.0)
	26.9	46.0	3.6

TABLE II

SOLUBILITY OF CADMIUM MANDELATE AT 25° C.

	PURIFIED CADMIUM MANDELATE SAMPLE USED FOR TEST	SOLUBILITY IN GRAMS PER 100 C.C. OF SOLUTION
Solution in distilled water adjusted to 25° C. from room temperature	No. 1	0.079
	No. 2	0.079
Solution in distilled water heated to 100° C and cooled to 25° C.	No. 2	0.078
Solution in acetic acid, pH 4.11, adjusted to 25° C. from room temperature	No. 1	0.089

The most convenient method of extraction for small samples of urine was found to be repeated shaking of the sample with ether in a 50 c.c. separatory funnel. Several solvents and mixtures of solvents, such as ether, chloroform, normal butyl alcohol, normal amyl alcohol, benzene, and carbon tetrachloride, were tried, but alcohol-free ether was found to be the best. When extracting with ether it was found necessary to avoid the use of grease on the stopcock of the separatory funnel, but the stopcock may be moistened with distilled water before the sample is introduced. Two satisfactory procedures were developed for transferring the extracted mandelic acid to a water solution preparatory to precipitation as the cadmium salt. In the semimicro procedure, the ether extract was neutralized with sodium hydroxide and the ether evaporated from the mixture. In the macro procedure, it was found expedient to extract the ether

solution with dilute sodium hydroxide and expel dissolved ether from the sodium hydroxide fraction by warming on the steam bath. By converting the mandelic acid to the sodium salt before evaporating the ether in both procedures all possibility of loss of the acid by volatilization was eliminated.

In order to avoid extracting unnecessary amounts of impurities from the urine samples by repeated shaking in the 50 c.c. separatory funnel, a series of extractions was made on 10 c.c. aliquot portions of a solution containing a known amount of mandelic acid. The results showing per cent recovery in these experiments are presented in Table III. It was found that five extractions are adequate.

TABLE III

ETHER EXTRACTION OF MANDELIC ACID FROM WATER
(0.2000 GM. MANDELIC ACID DISSOLVED IN 10 C.C. WATER)

NUMBER OF ETHER EXTRACTION	WEIGHT MANDELIC ACID RECOVERED GRAMS	RECOVERY PER CENT
3 × 30 c.c.	0.1768	88.4
5 × 30 c.c.	0.1989	99.5
7 × 30 c.c.	0.1998	99.9

METHOD OF ANALYSIS BY THE SEMIMICRO PROCEDURE

Reagents.—All chemicals used are Mallinekrodt reagents of the grade specified: Sulfuric acid A.R., diluted 1:1 by volume with distilled water; cadmium chloride solution, 50 Gm. cadmium chloride anhydrous A.R. in 100 c.c. water; sodium hydroxide 1N; chlorphenol red indicator, 0.1 per cent in 95 per cent ethyl alcohol; acetic acid glacial A.R.; ether anhydrous A.R.

Procedure.—Measure a 10 c.c. portion of the urine specimen into a 50 c.c. separatory funnel, add 1 c.c. of sulfuric acid (1:1), 30 c.c. anhydrous ether, and shake vigorously for three minutes. Allow the two layers to separate and withdraw the lower aqueous layer into a second 50 c.c. separatory funnel. Use necessary precautions to transfer all of the aqueous layer and ether-water suspension that does not separate readily. Wash the final drops from the stem into the second funnel with a stream of ether from a wash bottle. Transfer the ether layer remaining in the funnel into a 50 c.c. beaker; add 3 c.c. water, 5 drops chlorphenol red indicator, and a slight excess of 1N sodium hydroxide; and place on the steam bath with a current of air directed into the beaker. Allow all of the ether to evaporate thus leaving the mandelic acid in the aqueous layer as the sodium salt. While this first ether layer is evaporating, extract the aqueous solution in the second separatory funnel with another 30 c.c. of anhydrous ether and transfer this extract to the beaker in which the first extract was evaporated. Repeat the extraction and evaporation of the ether layer until five extractions have been made. The aqueous solution of mandelic acid in the beaker must be kept alkaline during these operations by the addition of sodium hydroxide, 0.5 to 1 c.c. at a time, as indicated by the chlorphenol red. Evaporate the aqueous solution of sodium mandelate thus obtained to about 3 c.c. and transfer quantitatively to a 10 c.c. micro beaker which has been previously weighed with a sintered Pyrex or porcelain filter stick.

Again evaporate the combined solution and washings on the steam bath to a volume of about 3 c.c., adjust the pH to approximately 5 by the addition of glacial acetic acid dropwise until the yellow color of the chlorphenol red indicator starts to appear, and finally add 0.4 c.c. of cadmium chloride solution. Evaporate the contents of the beaker to dryness on the steam bath, take up the residue in 3 c.c. of water, and allow the beaker and contents to stand for about thirty minutes with occasional agitation to obtain solution of the water-soluble salts. Filter the solution through the previously weighed filter stick. Rinse the inside of the beaker and filter stick with two 0.5 to 1.0 c.c. portions of water with successive withdrawal of each through the filter stick to remove all traces of soluble cadmium and sodium salts. Dry the beaker and filter stick in a vacuum desiccator over phosphorus pentoxide and weigh. Multiply the weight of cadmium mandelate by 0.7338 to convert to mandelic acid.

METHOD OF ANALYSIS BY THE MACRO PROCEDURE

Reagents.—The reagents are the same as those listed above for the semimicro method.

Procedure.—Any of the commonly used types of apparatus for continuous extraction by an immiscible liquid should suffice for this extraction. The type used here consisted of a 500 c.c. round bottom flask for a boiler, a 125 c.c. distillation flask for holding the sample, and a reflux condenser. Connections were made so that the condensed ether passed through a tube to the bottom of the flask holding the sample, overflowing through the side arm back to the boiling flask. The tube which returned the ether extended below the surface of the liquid in the boiler flask.

Introduce a 100 c.c. portion of the urine specimen and 10 c.c. sulfuric acid (1:1) into the distillation flask. Introduce 300 c.c. of anhydrous ether into the round-bottom flask. Connect the refluxing tube and the condenser to the two flasks, and connect the side arm of the distillation flask to the return tube so that excess ether will drain back into the round-bottom flask. Place the round-bottom flask on a steam bath or electric hot plate and allow the extraction to proceed for twelve hours, or overnight if convenient. At the end of the refluxing period, remove the round-bottom flask from the extraction setup, add 5 or 6 drops of chlorphenol red indicator and sufficient 1N sodium hydroxide to maintain the pink end-point of the indicator. Stopper the flask with a rubber stopper and shake vigorously for two or three minutes to insure that the end point has been reached. Place the round-bottom flask and contents on a steam bath and allow about one-half of the ether to evaporate. Transfer the ether-water mixture quantitatively to a 250 c.c. separatory funnel and add sufficient water to bring the total volume of the aqueous layer to 15 c.c. Shake the mixture vigorously for three minutes. Add more alkali, if necessary, to keep the aqueous layer alkaline. Withdraw the aqueous layer into a 250 c.c. beaker and rinse any adhering drops on the tip of the separatory funnel into the beaker with a stream of ether from the wash bottle. Repeat the extraction of the ether layer in the separatory funnel three more times, using 1 c.c. of 1N sodium hydroxide and 4 c.c. of water. Transfer each water extract to the same beaker. Place the

beaker on a steam bath and allow the ether to evaporate completely from the aqueous layer.

Adjust the pH of the water-extract to approximately 5 or 6 by the addition of sufficient glacial acetic acid to just reach the yellow end point of the chlorphenol red indicator. Precipitate the mandelic acid by adding 4 c.c. of cadmium chloride solution. Place the beaker containing the precipitate on a steam bath and allow the solution to evaporate to dryness. Add 10 c.c. of water to the residue, mix thoroughly, and allow to stand at room temperature for thirty minutes with occasional stirring. Filter the solution into a weighed sintered glass funnel, transferring the precipitate quantitatively into the funnel. Wash the precipitate with three 1 to 2 c.c. portions of water. Dry the funnel in a vacuum desiccator over phosphorus pentoxide and weigh. Multiply the weight of cadmium mandelate by 0.7338 to convert to mandelic acid.

DISCUSSION

The mandelic acid content of urine may be determined by either of the two procedures described above. In Tables IV and V are shown the results of experiments in which known quantities of mandelic acid were added to water and urine respectively and then recovered by the semimicro procedure. In Table VI are shown the results of experiments in which known quantities of mandelic acid were added to urine and recovered by the macro procedure. Although the methods appear to give equally satisfactory results over the range of concentrations studied, the semimicro method has the advantage that it requires considerably less elapsed time per analysis. It was found that five or six samples may be completed by the semimicroprocedure by an analyst in an eight-hour day. Another distinct advantage of the semimicromethod is that it permits the use of a smaller sample.

There is always an unavoidable uncertainty connected with the extraction of urine samples due to the lack of uniformity in the composition of different samples. It is conceivable that the cadmium mandelate procedure might be influenced by constituents in the urine, but significant interference by constituents of either normal or pathologic urine seems unlikely. A number of samples of urine originating from patients before the initiation of mandelic acid therapy were analyzed by the cadmium mandelate precipitation method, and in every case the complete absence of mandelic acid and all interfering impurities was indicated. Oxalic acid forms an insoluble cadmium salt, but it exists in urine in traces only. Hippuric acid is also only a minor constituent of human urine. The cadmium salts of benzoic acid and the common aliphatic acids are sufficiently soluble to avoid interference. Beta-hydroxybutyric acid, which may be present in urine under certain conditions, failed to yield a precipitate with cadmium chloride.

The results obtained by the cadmium mandelate precipitation method are subject to a slight error due to the solubility of the cadmium mandelate, but a high degree of accuracy is not required in a urinalysis and the solubility error is probably not significant except in samples where the mandelic acid concentration is very low. If an analysis required a more accurate determination the solubility error could be corrected either by calculation or manipulation.

TABLE IV

RECOVERY OF MANDELIC ACID FROM WATER

SEMIMICRO PROCEDURE

WEIGHT OF MANDELIC ACID ADDED TO 10 C.C. WATER GRAMS	WEIGHT MANDELIC ACID RECOVERED	
	TRIAL 1 GRAMS	TRIAL 2 GRAMS
0.0100	0.0119	0.0131
0.0500	0.0523	0.0521
0.1000	0.1037	0.1013
0.1500	0.1481	0.1514
0.2000	0.2015	0.1980

TABLE V

RECOVERY OF MANDELIC ACID FROM URINE

SEMIMICRO PROCEDURE

WEIGHT OF MANDELIC ACID ADDED TO 10 C.C. URINE GRAMS	WEIGHT OF MANDELIC ACID RECOVERED			
	URINE SAMPLE 1 GRAMS	URINE SAMPLE 2 GRAMS	URINE SAMPLE 3 GRAMS	URINE SAMPLE 4 GRAMS
0.0100	0.0105	0.0109	0.0093	0.0095
0.0500	0.0485	0.0508		
0.1000	0.1024	0.1049		
0.1500	0.1496	0.1511		
0.2000	0.2039	0.1995		

TABLE VI

RECOVERY OF MANDELIC ACID FROM URINE

MACRO PROCEDURE

WEIGHT OF MANDELIC ACID ADDED TO 100 C.C. URINE GRAMS	WEIGHT OF MANDELIC ACID RECOVERED			
	URINE SAMPLE 1* GRAMS	URINE SAMPLE 3* GRAMS	URINE SAMPLE 5 GRAMS	URINE SAMPLE 6 GRAMS
0.1000	0.1421	0.0911		
0.5000	0.5081			
1.000	1.011		0.953	0.977
1.500	1.569			
2.000	2.059			

*Same urine samples as reported in Table V.

From about one hundred and thirty analyses for mandelic acid in urine by the semimicro method performed in the course of a clinical investigation[2] only two specimens formed emulsions that could not be separated. Upon inquiring about the history of the specimens it was learned that they had originated from patients who had been given mercury therapy along with the sodium mandelate, which may have been responsible for the production of an emulsifying substance in the urine. These two specimens were easily extracted by a semimicro adaptation of the continuous extractor described in the procedure for the macro method. A 100 c.c. round-bottom flask and a 50 c.c. distilling flask were used in the setup. Recovery experiments on known quantities of mandelic acid added to normal urine established the fact that four to five hours was sufficient time for the continuous extraction of 10 c.c. samples.

In Table VII is shown a series of results on case specimens of urine in which the samples were analyzed for mandelic acid by both the semimicro and macro procedures.

The isolation and determination of mandelic acid as cadmium mandelate may be of value in the analysis of other solutions or mixtures such as elixirs or tablets.

TABLE VII

COMPARISON OF CASE SPECIMENS BY SEMIMICRO AND MACRO METHODS

URINE SPECIMEN	WEIGHT OF MANDELIC ACID FOUND PER 100 C.C. URINE	
	SEMIMICRO GRAMS	MACRO GRAMS
No. 1	0.19	0.20
No. 2	1.62	1.64
No. 3	0.35	0.32
No. 4	0.12	0.11
No. 5	0.03	0.04

SUMMARY

A method for the determination of mandelic acid in urine is presented and its use for other types of samples is suggested. The procedure is based on the extraction of the acid with ether, conversion to the sodium salt, and precipitation as cadmium mandelate. A semimicro and a macro procedure are given and two methods of extraction are described. The methods are accurate and require no special equipment or reagents. Several determinations may be carried out simultaneously.

The composition of cadmium mandelate has been confirmed by analysis and its solubility redetermined. A method is reported for the determination of cadmium as the oxide simultaneously with the determination of carbon and hydrogen by combustion.

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A RAPID AND SIMPLE TECHNIQUE FOR THE DETERMINATION OF GELATIN*

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THIS report deals with a technique for the determination of gelatin protein in a mixture of blood and gelatin or urine and gelatin in vivo as well as in vitro. The technique described here is merely the application of well-known methods and principles of chemistry in the separation of plasma and gelatin or urine and gelatin proteins. The present paper is reported because of the increased interest in gelatin as a blood substitute and because of the absence in the literature of suitable simple methods for the determination of gelatin.[1]

The basis for the separation of gelatin from plasma proteins is the property of gelatin protein in 95 per cent ethyl alcohol and in 5 per cent trichloracetic acid. (Gelatin throughout this paper refers to an autoclaved solution.) The addition of 95 per cent alcohol to a gelatin solution results in the protein of the gelatin being precipitated, the nonprotein constituents remaining in the supernatant fluid after centrifugation. The addition of 5 per cent trichloracetic acid to a gelatin solution does not precipitate the gelatin proteins. It is a well-known fact that 5 per cent trichloracetic acid or 95 per cent alcohol precipitates the proteins of serum or plasma, the nonprotein constituents remaining in solution.[2] Thus in a mixture of gelatin and plasma, the gelatin may be separated from the plasma by the precipitation of plasma proteins with trichloracetic acid, thereby leaving the nonprotein constituents of both plasma and gelatin and the gelatin protein in the supernatant fluid. Every sample of gelatin had a nonprotein nitrogen content varying between 30 and 75 mg. per cent after autoclaving. Prolonged autoclaving increased the nonprotein nitrogen and decreased the viscosity of the solution. A standardized procedure of autoclaving was used therefore. Now, if a total nitrogen determination is made by the micro-Kjeldahl method on the 5 per cent trichloracetic supernatant fluid and the nonprotein nitrogen is determined by the usual nesslerization method on an alcoholic filtrate of a plasma-gelatin mixture, the protein nitrogen of gelatin may be obtained by subtracting the nonprotein nitrogen from the total nitrogen. The protein nitrogen multiplied by the protein factor for gelatin of 5.25 yields the protein gelatin in the mixture.

The plasma protein of the mixture may be obtained by analyzing the gelatin-plasma mixture for total nitrogen, and then subtracting the total nitrogen of the 5 per cent trichloracetic acid supernatant which contains the gelatin protein nitrogen and the nonprotein nitrogen constituents of both gelatin and plasma. This figure represents total plasma protein nitrogen, which multiplied by the usual protein factor of 6.25 yields the protein of the plasma. It is obvious that

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by adding the plasma protein to the gelatin protein the true combined total protein of the gelatin-plasma mixture is obtained.

These same principles may be applied to gelatin-urine analysis. In passing, it should be pointed out that the qualitative tests for protein in urine, such as 5 per cent acetic acid and sulphosalicylic acid, are not suitable for the detection of gelatin, since gelatin is not precipitated by either of these reagents.

EXPERIMENTAL

Ossein or pigskin gelatins prepared in 0.85 per cent NaCl solutions, autoclaved for one-half hour to one hour at 256° F., were used in vitro and in vivo experiments. In vitro experiments were performed by mixing gelatin and plasma in varying amounts. Total proteins were determined on the gelatin and plasma before the mixtures were made; the mixtures were analyzed for gelatin and plasma proteins, and the theoretical values were calculated. In Table I are represented the theoretical and recovered gelatin and plasma protein values.

Total nitrogen determinations were made by diluting 0.5 c.c. of the plasma-gelatin mixture to 25 c.c. with 0.9 per cent NaCl and analyzing 1 c.c. aliquots for total nitrogen by the micro-Kjeldahl method of Ma.[3]

Gelatin nitrogen was determined by precipitating the plasma proteins contained in 0.5 c.c. of the gelatin-plasma mixture with 7 c.c. of 5 per cent trichloracetic acid and by analyzing 1 c.c. portions of the supernatant for total nitrogen (G.N.) by the micro-Kjeldahl method.

Nonprotein nitrogen (N.P.N.) was determined by precipitating the gelatin and plasma proteins in 0.5 c.c. of the mixture with 7 c.c. of 95 per cent alcohol, determining the amount of N.P.N. in 5 c.c. of the supernatant by the usual methods using direct nesslerization.[4]

Protein values were obtained by the following formulas:

$(\text{T.N.} - \text{G.N.}) \times 6.25 =$ Plasma protein in grams per 100 c.c.

$(\text{G.N.} - \text{N.P.N.}) \times 5.25 =$ Gelatin protein in grams per 100 c.c.

Gelatin protein plus plasma protein = Combined protein of mixture.

In vivo experiments were performed on dogs infused with known quantities of gelatin. Gelatin and plasma protein analyses on the blood of these animals one hour, twenty-four hours, forty-eight hours, seventy-two hours, and five days later indicated that gelatin left the circulation very rapidly.[5] The analyses were made in duplicate and the values obtained agreed within ±3 per cent error.

In order to rule out the rapid loss of gelatin from the circulation due to diffusion of gelatin into the red cells, heparinized blood was mixed with equal amounts of gelatin solution. The mixture was gently shaken for one hour, and then centrifuged, with the plasma analyzed for gelatin and plasma protein. In one experiment the red blood cells of the mixtures were repeatedly washed with 0.85 per cent NaCl; the washed cells were then frozen rapidly in carbon dioxide snow and alcohol, thawed rapidly, and analyzed for gelatin. Table II represents the results of this experiment. The theoretical values were calculated from hematocrit determinations of the blood before adding the gelatin.

DISCUSSION

From the data presented in Tables I and II, it would appear that the technique for the determination of gelatin and plasma protein in a mixture of gelatin

TABLE I

RECOVERY OF GELATIN FROM A MIXTURE OF PLASMA AND GELATIN

PROPORTION GELATIN: PLASMA	PER CENT PROTEIN CONTENTS		PER CENT GELATIN PROTEIN			PER CENT PLASMA PROTEIN			PER CENT COMBINED PROTEIN			NPN		PER CENT OF GELATIN RECOVERED
	GELATIN	PLASMA	CALCUL.	RECOVER.	DIFFER.	CALCUL.	RECOVER.	DIFFER.	CALCUL.	RECOVER.	DIFFER.	CALCUL.	RECOVER.	
1:1	6.64	4.68	3.32	3.26	−.06	2.34	2.47	+.13	5.66	5.73	+.07	64	62	98.2
1:2			2.23	1.98	−.25	3.12	3.16	+.04	5.35	5.14	−.21	50	52	88.8
1:4			1.34	1.21	−.13	3.74	3.96	+.22	5.08	5.17	+.09	38	32	90.3
1:9			0.67	0.63	−.04	4.21	4.02	−.19	4.88	4.65	−.23	30	27	94.0
2:1			4.45	4.39	−.06	1.56	1.70	+.14	6.01	6.09	+.08	78	75	98.6
1:1	4.80	5.94	2.40	2.21	−.19	2.97	3.21	+.24	5.37	5.42	+.05	79	80	92.0
1:2			1.60	1.46	−.14	3.96	4.20	+.24	5.56	5.66	+.10	81	80	91.2
1:4			0.96	0.89	−.07	4.75	4.79	+.04	5.71	5.68	−.03	83	86	92.5
3:2			2.87	2.65	−.22	2.38	2.67	+.29	5.25	5.32	+.07	78	76	92.3
1:1	6.88	6.56	3.44	3.21	−.23	3.28	3.49	+.21	6.72	6.70	−.02	48	40	93.3

TABLE II

RECOVERY OF GELATIN FROM A MIXTURE OF BLOOD AND GELATIN

PROPORTION GELATIN : BLOOD	PER CENT GELATIN PROTEIN			PER CENT PLASMA PROTEIN			PER CENT GELATIN RECOVERED	PER CENT PROTEIN CONTENTS		BLOOD HEMATOCRIT PER CENT RED BLOOD CELLS
	CALCULATED	RECOVERED	DIFFERENCE	CALCULATED	RECOVERED	DIFFERENCE		GELATIN	PLASMA	
1:1	4.66	4.27	-.39	1.82	2.12	+.29	91.7	5.54	6.98	50
1:1	4.41	3.80	-.60	1.80	2.23	+.43	86.2*	6.88	5.02	44.6

*Analysis of washed, hemolyzed red blood cells was negative for gelatin.

and plasma is adequate for biologic purposes, the differences between the expected and the obtained values being within the limits of error of the method. Due to the viscous nature of gelatin, difficulty is encountered in adequately draining pipettes when measuring gelatin. This may in part explain the loss of gelatin in the recovery experiments, since the pipettes containing plasma and gelatin cannot be rinsed out with trichloracetic acid. When blood and gelatin were mixed, the gelatin recovery was 91.7 and 86.2 per cent. Analysis of saline washed blood cells hemolyzed on freezing and thawing (86.2 per cent recovery experiment) was negative for gelatin. These data would indicate that no, or at most insignificant, amounts of gelatin entered the red blood cells.

After the writing of this paper the work of Little and Wells appeared.[6] These authors employed sodium tungstate as gelatin precipitant and 10 per cent trichloroacetic acid as plasma protein precipitant. No recovery data were supplied.

SUMMARY

1. A technique applying known methods has been described for the determination of gelatin in a mixture of gelatin and plasma.

2. Recovery experiments indicate the method suitable for biologic purposes.

3. The technique described is also applicable to urine and gelatin analysis.

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MEDICAL ILLUSTRATION

THE CONTROL OF EXPANSION AND CONTRACTION OF RUBBER CASTS*

CARL DAME CLARKE, AND FREDERICK MATTHEW STINER, WASHINGTON, D. C.

SHRINKAGE of rubber prostheses of facial and body members has presented a definite problem. Rubber shrinks slightly on setting (coagulation) and drying. This shrinkage may be great or infinitesimal. Nevertheless, it may be sufficient to cause an improper fit of the artificial part to the human tissues. Therefore the following experiments were carried out in an effort to overcome this difficulty. It was found possible to expand a rubber cast to more than three times its original surface area or slightly less than two diameters by soaking it in benzine. Likewise, it was discovered how to reduce or shrink it to a fraction of its original size. The ability to reduce the size has its place in art and medicine as well as the need for enlarging to offset shrinkage. A practical example of where shrinkage may be a necessity rather than a fault is when a rubber hand is to be made of a proper size to fit a particular individual who has lost his hand. These hands are invariably molded from a second individual (donor) for supplying a cast to the first (recipient).

Both expansion and contraction must take place without an appreciable loss of detail. Therefore, coins having a wealth of detail were chosen for these experiments. Indeed, the microscopic writing on one coin (Figs. 1 and 2) became plainly visible to the unaided eye on enlarging. It was found that in the expansion of a prosthesis the distortion often took place in proportion to the thickness of the rubber. The thinner parts became distorted more rapidly. However, this was offset in the drying of the expanded rubber to a greater extent than had taken place during the expansion. As a result the prosthesis assumed an accurate but enlarged pattern that was satisfactory for recasting into a final prosthesis for use on the patient.

Briefly, the expansion is accomplished by soaking the vulcanized rubber pattern in benzine until it has expanded to a degree slightly above that which is desired. It is then allowed to shrink somewhat to assume correct but enlarged proportions. The pattern is then used for plaster molding for the final rubber prosthesis or cast.

Expansion and contraction are also governed by the amount of filler (clay) in the rubber. The more filler, the greater the contraction. However, expansion takes place to a lesser degree if the cast is filled.

According to the record kept of experiments, the following results in expansion were noted. These experiments were done on rubber casts from plaster molds.

*From the Army Medical Museum.
Received for publication, March 1, 1943.

EXPERIMENT 1

Subject: *Coin.*

Materials: Vultex H-235 2 parts — Dixie clay 1 part

Expanding agent: Benzine

Minutes	Size in mm.	Expansion gain in mm.
0	40	0
45	55	15
65	58	18
80	60	20

This cast reached its maximum expansion of 60 mm. in 80 minutes.

EXPERIMENT 2

Subject: *Coin.*

Material: Vultex F-293 2 parts — Water 1 part

Expanding agent: Benzine

Minutes	Size in mm.	Expansion gain in mm.
0	40	0
15	60	20
60	70	30

From this experiment it will be noticed that the unfilled cast expanded more rapidly than the filled cast. In 15 minutes it reached 60 mm. in diameter, a 20 mm. gain. It reached its maximum expansion of 70 mm. in 60 minutes.

EXPERIMENT 3

Subject: *Coin.*

Material: Vultex H-235 2 parts — Dixie clay 1 part

Expanding agent: Benzine

Minutes	Size in mm.	Expansion gain in mm.
0	40	0
5	42	2
10	44	4
15	46	6
30	50	10
45	53	13
60	60	20
225	60	20

This cast reached its maximum expansion in 60 minutes. However, soaking was continued to see whether disintegration would take place. This became apparent as soaking continued over exceptionally long periods of time. Vulcanization plays a large part in the degree of disintegration. Unvulcanized casts disintegrate rapidly. The well-vulcanized cast holds up after prolonged soaking in benzine.

EXPERIMENT 4

Subject: *Coin.*

Material: Vultex F-293

Expanding agent: Benzine

Minutes	Size in mm.	Expansion gain in mm.
0	40	0
5	43	3
10	46	6
15	46.5	6.5
30	49	9
45	52	12
60	55	15
75	57	17
90	62	22

This cast was slightly thicker than the cast used in Experiment 2, therefore expansion took place more slowly.

EXPERIMENT 5

Subject: Prosthetic Nose.

Material: Vultex H-235 2 parts — Dixie clay 1 part

Expanding agent: Benzine

Minutes	Size in mm.	Expansion gain in mm.
0	74 high, 53 wide	0 high, 0 wide
24	82 high, 62 wide	8 high, 9 wide
45	85 high, 65 wide	11 high, 12 wide

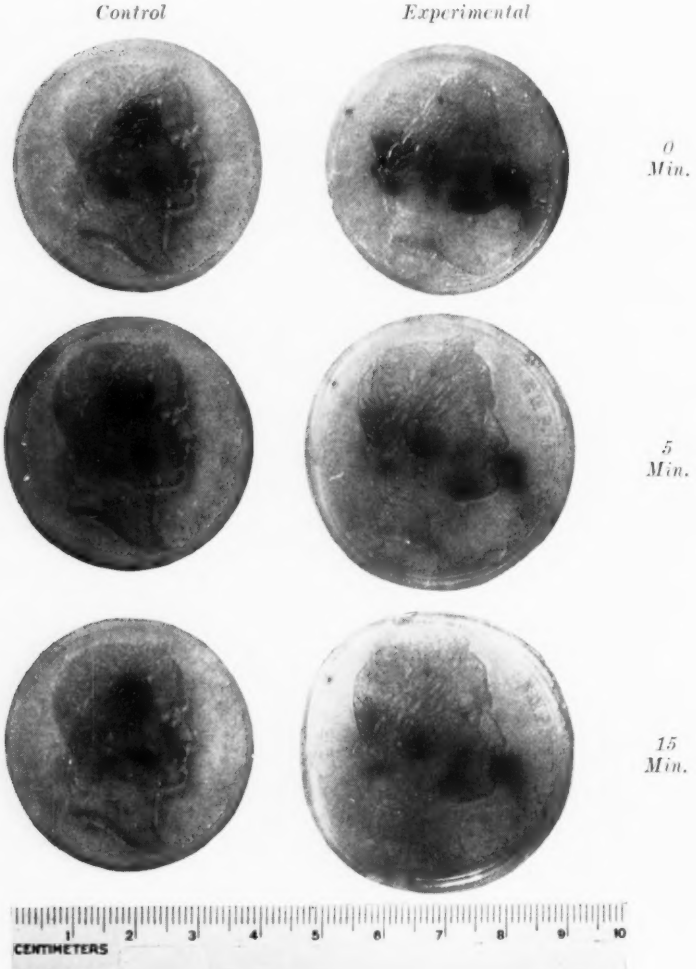

Fig. 1.—The control and experimental of vulcanized latex containing no filler. It will be noticed that after 15 minutes of soaking in benzine distortion begins to appear.

After 45 minutes in the benzine distortion began to take place, so the prosthesis was removed from the benzine. On examination it was found that expansion had taken place rapidly in the thin area. When the prosthesis was allowed to stand in the air it was found that on evaporation of the benzine shrinkage took place more rapidly in the thin areas than in the thick ones. Therefore, the model shrank back to a true ratio in the proportionate areas but there was an enlargement throughout the entire cast. The photograph in Fig. 3 was taken five minutes after removal from the benzine. It shows clearly the proportional

enlargement that is possible. This enlargement is greater than is necessary for prosthetic appliances because the natural shrinkage of rubber is not sufficient to offset such an enlargement. Although it may be necessary to enlarge prosthetic hands and arms beyond the actual size of the donor's hand to provide the

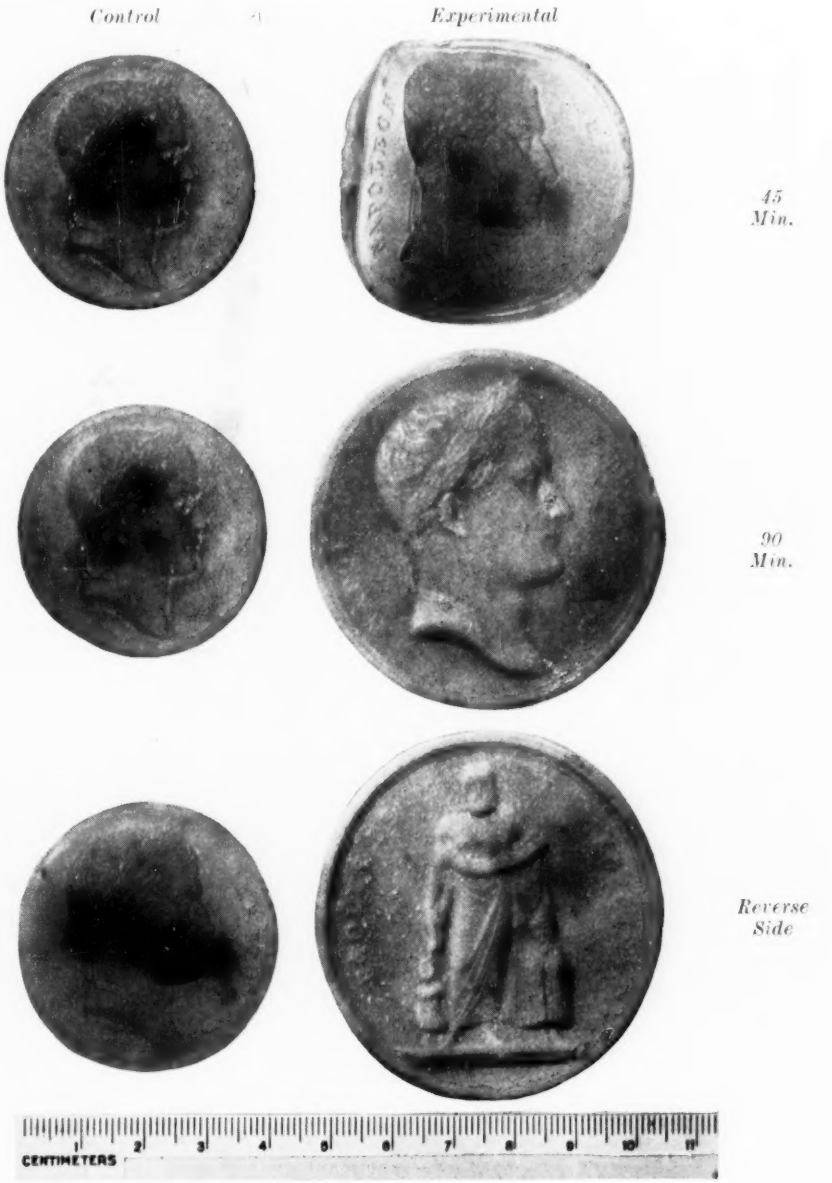

Fig. 2.—This is a continuation of the illustration seen in Fig. 1. Distortion has taken place rapidly in 45 minutes of soaking. At the end of 90 minutes the experimental has flattened without distortion. The bottom picture shows the reverse side of the coin.

correct size for the recipient, it is not likely that an enlargement of such proportions would be necessary in many cases. However, greater enlargements in true ratio and proportions are possible.

As mentioned previously, a distorted enlargement of greater proportions

was obtained in 45 minutes' soaking in benzine. Five minutes after it was removed from the benzine the prosthesis shrank to true but still enlarged proportions to the control. As the benzine continued to evaporate the prosthesis became smaller and eventually returned to the same dimensions as the control. This took about two hours. At any point during this shrinkage the model could have been remolded to obtain a permanent prosthesis of enlarged proportions that would not shrink appreciably regardless of time.

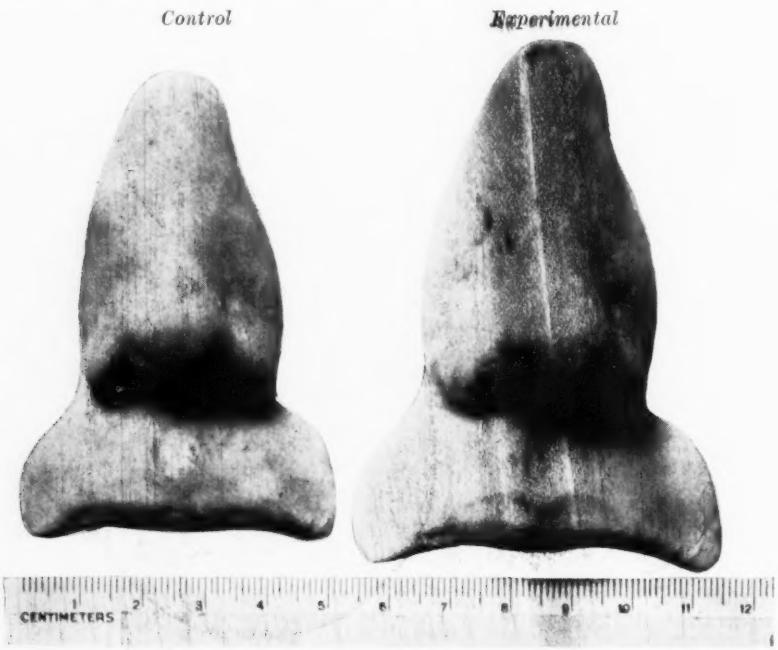

Fig. 3.—Rough casts of filled rubber prosthesis showing expansion in true proportions. This has expanded more than necessary, since it is only essential to offset normal shrinkage for this type of prosthesis. Filled rubber shrinks about 2 mm. to 40 mm.

Distortion.—Upon further investigation it was found that the experimental eventually assumed true proportions when it reached the saturation point during soaking in the benzine. Enlargement of nearly two diameters or roughly three times in area was obtained. The more filler used in the rubber latex the smaller was the enlargement. Nevertheless, this had little effect on distortion. As the enlarging process advanced the model lost physical strength but it always possessed enough toughness to be molded in plaster at any time during the soaking in benzine. In prosthetic hands the expansion can be held permanent for a longer period of time or shrinkage prevented by filling the rubber shell with a microcrystalline wax. This wax is pliable and permits the fingers to be moved in any position desired before molding. A one-piece plaster mold is then made of the rubber hand. After the plaster mold has set the wax is melted out. The collapsible rubber pattern shrinks and is finally pulled from the mold. This one-piece mold is used for pouring duplicate rubber hands to be attached to the patient. In this way prostheses without seam lines are obtained. This technique will be described more fully in a future article.

Accelerated Shrinkage.—Contrary to common belief it should be realized that rubber can be cast in practically any type of mold. This includes molds of agar, plaster, wood, cement, terra cotta, glass, rubber, and synthetic plastics.

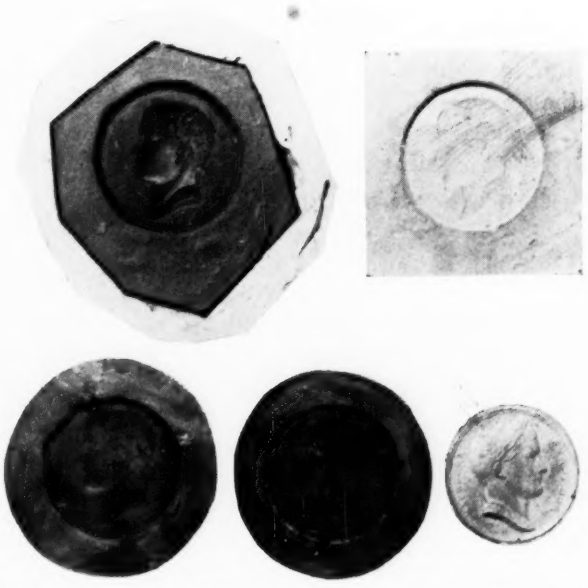

Fig. 4.—Rubber casts can be made from many types of molds. *Upper left:* An agar mold. *Upper right:* A plaster mold. *Lower left:* A wax mold. *Center:* A rubber mold. The small impression at the lower right is a rubber cast.

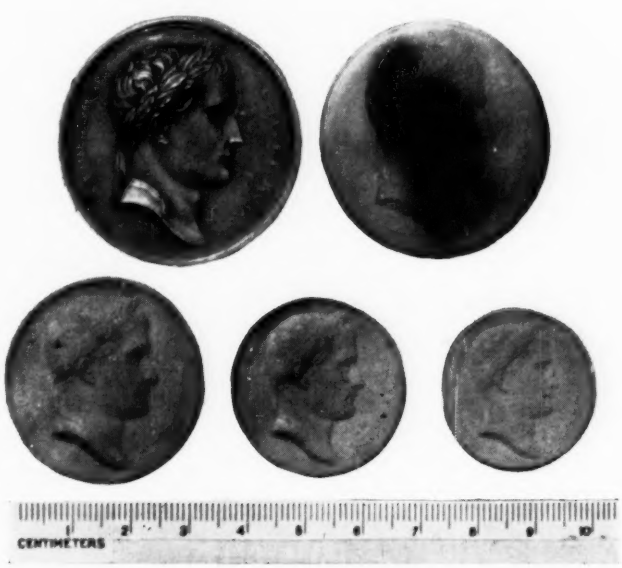

Fig. 5.—*Top left:* Original coin. *Top right:* First cast from a plaster mold. This cast contained no filler. It was used as a control. *Lower left:* The first cast from an agar mold showing a reduction. *Center:* A second recasting from an agar mold showing further reduction. *Lower right:* A third recasting showing a still further reduction. These recastings were of filled rubber and photograph better than the unfilled rubber which was nearly transparent.

In fact, the problem has never been with the molding material, but new separating mediums had to be devised to suit the mold. Rubber adheres to rubber, therefore soap and a salt acting as a coagulating agent for rubber had to be used on the mold. Rubber will not stick to agar. However, a coagulating salt had to be added to the agar mix to coagulate the rubber latex on the surface of the agar to build up a sufficient thickness of rubber to make the cast of practical use. The subject of separating and coagulating agents also will be discussed further in a future article. This subject has been considered in some detail in the book *Molding and Casting*.[1]

Shrinkage takes place more readily in casts that dry from the side next to the mold. Those that dry from the hollow side of the cast only, shrink less. For example, when rubber is poured into a dry plaster mold, water is absorbed from the latex mix into the porous mold. If rubber is poured into an agar, a rubber or synthetic plastic mold which is nonabsorbent, a rubber coating or layer must be built upon the mold. The excess then is poured out. This coagulated layer of rubber must dry from the hollow side. Furthermore, after coagulating it must not adhere to the surface of the mold. Both the inner and outer surfaces are exposed to the air and shrinkage takes place rapidly. This shrinkage can be offset to some extent by pouring over the coagulated and partially dried rubber layer a microcrystalline wax which presses the rubber back to the mold surface.

If a filled rubber latex is poured into an agar mold, proportional contraction or shrinkage readily occurs on the drying of the rubber. An agar mold will also quickly contract on drying. Here considerable distortion takes place in the mold. This distortion can be prevented by the addition of sorbitol to the agar mix. Shrinkage follows but it is not distorted contraction. In the first casting of a filled rubber latex positive in an agar mold containing no sorbitol a proportional shrinkage of 5 to 40 millimeters occurs, thus producing a cast of 35 millimeters in diameter. The second casting from a second agar mold of the first casting shrinks from 35 to 30 millimeters, and the third casting shrinks to 27 millimeters. Therefore, three castings into agar molds produce a proportional shrinkage of 13 millimeters. As a result, the final cast is reduced to nearly half a diameter of the original pattern. The same process can be continued without appreciable loss of detail.

Prinz[2] gives the following formula for reducing the size of plaster of Paris casts:

Alcohol	1 part by weight
Water	2 parts by weight
Plaster of Paris	enough to suit

The cast is allowed to dry for a few days in a warm place. After the alcohol has been evaporated completely, the finished cast will be about $\frac{1}{25}$th smaller in all directions than the original model from which the impression was made. By repeating the process any desired reduction may be obtained.

The reduction of only $\frac{1}{25}$th in this case is so small that the experiment was not done. At this rate many castings would be necessary to obtain the $\frac{1}{8}$th reduction possible with one casting of rubber into agar. However, from a research standpoint Prinz's method may prove of value.

[1]Clarke, Carl D.: Molding and Casting, Baltimore, 1938, John D. Lucas Co.
[2]Prinz, Herman: Dental Formulary, Lea & Febiger, Philadelphia, 1930.

BOOK NOTICES

Fundamentals of Immunology*

UNTIL within the last decade medical writers have approached the subject of immunology chiefly through the medium of bacteriology. Most of the leading texts on the subject have dealt with bacteriology and immunology. Recent advances in the study of the nature of antibodies and the mechanism of their reaction with foreign cellular and noncellular proteins have been made especially through the medium of protein chemistry and physical chemistry. Dr. Boyd's book, "Fundamentals of Immunology," presents an adequate discussion of recent advances in this new phase of the study. It may be highly recommended as a reference volume on the mechanism of antigens, antibodies, and their interactions, and it is so constructed that it will also serve as a textbook and laboratory manual. The discussion of anaphylaxis is rather brief. That of clinical allergy is decidedly superficial, but this phase of the discussion was not the purpose of the book.

The discussion of antigen-antibody reactions includes study of blood groups, complement and complement fixation, and immune reactions to bacteria, viruses, and parasites.

Physiological Regulations†

DOCTOR Adolph's monograph, *Physiological Regulations*, deals chiefly with the study of the mechanism maintaining water balance in experimental animals and in man. It is highly technical and should be of chief interest to physiologists and biologists.

Familial Nonreaginic Food-Allergy‡

DR. COCA, like many other allergists, has long been conscious of the inadequacy of skin tests with food extracts. His new book, *Familial Nonreaginic Food Allergy*, describes a new approach to the study of allergenic foods. Some food extracts produce positive skin reactions which are truly reaginic. Others appear to give false negative reactions. Dr. Coca believes that these are nonreaginic. His experience has been that this type of food, when eaten by one who is sensitized to it, produces an increase in the pulse rate which can be used diagnostically.

Dr. Coca documents his thesis with detailed presentation of case records. He describes the technique of diagnostic study in detail, and outlines the program of practical management.

Irrespective of whether the author's theory concerning the effect of foods on the pulse rate will be substantiated by others, the volume covers the careful investigations of a leader in the field of allergy and will be of interest not only to allergists, but to internists and others.

Psychosomatic Medicine§

PSYCHOSOMATIC medicine may be termed the newest specialty in the field of internal medicine and psychiatry. The psychiatrist has in the past dealt with psychoties, many of whom are incurable, but has recognized the field of functional nervous disorders in which

*Fundamentals of Immunology. By William C. Boyd, Ph.D., Associate Professor of Biochemistry, Boston University School of Medicine; Associate Member, Evans Memorial, Massachusetts Memorial Hospitals, Boston, Mass. With 45 Illustrations. Cloth, 446 pages, $5.50. Interscience Publishers, Inc., New York, N. Y., 1943.

†Physiological Regulations. By Edward F. Adolph, Associate Professor of Physiology in the University of Rochester. Cloth, 502 pages, $7.50. The Jaques Cattell Press, Lancaster, Pa., 1943.

‡Familial Nonreaginic Food-Allergy. By Arthur F. Coca, M.D., Medical Director, Lederle Laboratories. Cloth, 160 pages, $3.00. Charles C Thomas, Publisher, Springfield, Ill., and Baltimore, Md., 1943.

§Psychosomatic Medicine. The Clinical Application of Psychopathology to General Medical Problems. By Edward Weiss, M.D., Professor of Clinical Medicine, Temple University Medical School, Philadelphia, and O. Spurgeon English, M.D., Professor of Psychiatry, Temple University Medical School, Philadelphia. Cloth, 687 pages, $8.00. W. B. Saunders Company, Philadelphia and London, 1943.

supervised orientation may relieve the patient. It is in this group that the Freudian procedures of psychoanalysis and psychotherapy, used in the broad sense, are often helpful. The internist, on the other hand, treats many cases with organic disease in which he realizes that emotional factors play a part but which he is unable to study adequately because of too limited experience in psychotherapy. Collaborative efforts by the psychiatrist, or psychologist, and the internist may be expected to solve many of these patients' problems. Psychosomatic medicine may be loosely described as the treatment of emotional factors which color the symptomatology of somatic or organic disease.

The volume *Psychosomatic Medicine* results from the collaborative effort of an internist and a psychiatrist. The first two chapters deal with presentation of the problem, with description of illustrative cases. Reading these chapters, one will agree enthusiastically with the authors as to the need for a new method of treatment for such cases and is likely to conclude that the procedure should be very simple, just a matter of horse sense. The last four chapters describe the technique of psychosomatic study and treatment in detail. After reading them, one realizes that this is a highly technical field requiring extensive training, if the therapist wishes to do good rather than harm. Although psychosomatic practice will necessarily develop into a strict specialty, every physician practicing medicine should be well acquainted with the problems and methods in the field. This volume fills this need adequately. In it one finds discussions of the psychosomatic factors in cardiovascular disease, disorders of the gastrointestinal system, the endocrine system, the respiratory system, allergic diseases, the central nervous system, the genitourinary system, and disorders of sexual function. There is also a chapter on military medicine.

Man in Structure and Function*

MAN in Structure and Function is a two-volume semipopular discussion of human anatomy and physiology written by a German physician and translated by Dr. George Rosen. The text is free enough from technical terminology and complicated verbiage so that a well-educated layman, not averse to making the acquaintance of some new words, can read it with understanding. The fascinating part of the book, however, is its illustrations. These might be termed "inspired." They illustrate statements concerning the structure and especially the function of the body in the form of comparisons with familiar objects and experiences which all readers can understand with no difficulty at all. There are 461 of them. These volumes are worth their price for the illustrations alone.

*Man in Structure and Function. Volumes I and II. Translated from the German and edited by George Rosen, M.D. Cloth; Vol. I, 341 pages; Vol. II, 742 pages; $10.00. Alfred A. Knopf, New York, 1943.

COMMUNICATION

The first Women's Army Auxiliary Corps Hospital Unit, organized to replace able-bodied men in laboratories and hospitals of Army posts and stations, is now serving with the Army at Station Hospital, Fort Oglethorpe, Ga. This group of medical, surgical, and laboratory technicians, x-ray technicians, dental assistants, pharmacists, medical stenographers, clerks, and typists is expected to be the forerunner of other WAAC hospital units to be organized and sent out to Army posts. WAACs are also serving as technicians, hospital mess sergeants, cooks, and chauffeurs.

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